Sex determination, adaptive divergence, and the role of inversions in ecotypes of the intertidal snail *Littorina saxatilis*

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DECLARATION

I, the author, confirm that the Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not previously been presented for an award at this, or any other, university.

Chapter Two has been published as a short communication *in the journal* 'Evolution Letters' *with the co-authors Eva L Koch, Sean Stankowski, Roger K Butlin, Rui Faria, Kerstin Johannesson and Anja M Westram, and is available at doi:10.1002/evl3.295. The published version is included as Chapter Two but with altered formatting to maintain consistency and pagination with the rest of the thesis; see DOI for published formatting. Contributions of authors in this chapter and Chapters Three and Four are detailed in Chapter One (General Introduction).*

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THESIS SUMMARY

Sex determination and sex chromosomes exhibit great diversity within and between species, in their mechanisms and drivers, and over space and time. Why and how this variation has evolved remains poorly understood and empirical evidence remains scarce. Species with young, emerging sex determination systems are useful tools for understanding these processes, in particular in species with labile sex determination between environments. The role of ecology in sex-specific selection is unclear but likely to be central to these processes. Understanding the drivers and mechanisms for adaptive divergence, reproductive isolation and sex determination in the same populations will be crucial and allows the interface between these processes to be tested. In this thesis, I investigate the above processes in the intertidal snail, *Littorina saxatilis*, utilising a combination of genomic approaches based on previous field sampling and manipulative experiments in natural populations. I uncover evidence for a multigenic female-heterogametic sex determining system in operation in multiple populations that involves genomic regions on two linkage groups and multiple sex-linked inversions. I characterise patterns of sex- and ecotype-specific genomic associations of SNPs and inversions, revealing the role of habitat-dependent sex-specific selection and the concurrent role of inversions in the differentiation of both sexes and ecotypes. Through experimental testing, I quantify differential survival and movement of locally adapted ecotypes across habitats, specifically evidencing the role of divergent selection and habitat choice in reproductive isolation between ecotypes. I further show that arrangement frequencies of two sex-linked inversions are mediated by sex-, ecotype-, and habitat-based selective interactions. Together, my findings shed light on drivers and mechanisms of sex determination, ecotype divergence, and the role of inversions in *L. saxatilis.* More generally, they offer insight into the relationship between sex- and environment-based selection and transitions in sex-determination systems across heterogeneous environments, uncovering an ideal system for future work.

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CHAPTER ONE
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General Introduction

1.1 Sex determination and sex chromosomes: diversity, drivers, and mechanisms

The huge diversity of sex chromosomes and mechanisms of sex determination across the natural world has been a topic of debate and research for many years. Biological sex is a fundamental part of natural systems which affects all aspects of life, including evolution, behaviour, genetics and physiology, yet has no conserved mechanism for determination (Bachtrog *et al*. 2014; Furman *et al*. 2020). It is determined though a multitude of systems both within and between species, which may be environmental or genetic or a combination of the two (Bachtrog *et al*. 2014). When sex is determined genetically, which will be the focus here, sex chromosomes are often involved (Rice 1984b). Similar mechanisms have also repeatedly evolved from independent or parallel origins (Wright *et al*. 2016; Montiel *et al*. 2017; Nacif *et al*. 2022). Research into these areas has met with difficulty due to a combination of factors, meaning the drivers and mechanisms of the evolution of sex determination and sex chromosomes remain poorly understood (Pennell *et al*. 2018; Vicoso 2019; Ramos and Antunes 2022).

A common hypothesis for the driver of sex chromosome evolution is focussed around a role of sexual antagonism (Fisher 1931; Rice 1987; Rice 1996; Wright *et al*. 2016), although alternative models exist (e.g., Lenormand and Roze 2022). Genomic conflict between the sexes arises when sex-specific patterns of selection result in traits having differing optima in each sex, so alleles at relevant loci have opposing effects on fitness in each sex due to the shared genome (Connallon and Clark 2014; Mank 2017). Sexually antagonistic loci are frequently found on sex chromosomes; linkage of sexually antagonistic loci to a sex-determining (SD) locus resolves the genomic trade-off and facilitates separate evolution of the sexes and sexual dimorphism (Wright *et al*. 2016). Therefore, sexually antagonistic loci may drive the evolution of sex chromosomes themselves since methods of recombination suppression are selected for when sexually antagonistic loci are in proximity to a SD locus (Charlesworth 2017). However, as these recombination-suppressed regions around SD loci are the most advantageous place in the genome for sexually antagonistic loci, they may accumulate on pre-existing sex chromosomes (Rice 1984b; Charlesworth 2016).

Sex determination and sex chromosome evolution are highly dynamic (Bachtrog *et al*. 2014; Katsumi *et al*. 2022). Even once a mechanism has become established in a species or population, switches in sex determination systems and turnovers of sex chromosomes are common in some lineages and can occur repeatedly within groups (Bachtrog *et al*. 2014; Pennell *et al*. 2018). Once more, the drivers of such transitions are an area of uncertainty, but fitness differences between the sexes (potentially due to sexually antagonistic loci), selection on sex ratio (including due to selfish genetic elements), and neutral processes may all contribute (Beukeboom and Perrin 2014). Genetic sex determination can also be polygenic, and SD loci may be spread across the genome rather than on one chromosome, such that multiple genomic regions are important and show evidence of genetic sex differences (Yusa

and Kumagai 2018; Moore *et al*. 2022; Ramos and Antunes 2022). Switches in sex determination can involve a master SD locus being overtaken by a different locus further down in the polygenic sex determination cascade (Bachtrog *et al*. 2014).

1.2 Spatially heterogeneous sex-specific selection and reproductive isolation

Species that inhabit spatially heterogeneous environments offer further evidence for variation in sex determination processes and can be useful tools for understanding selective drivers (Abbott *et al*. 2017; Furman *et al*. 2020). Populations of species have been shown to differ in their sex chromosomes and SD mechanisms (Kitano *et al*. 2009; Miura *et al*. 2012; Yoshida *et al*. 2014; Smith *et al*. 2016; Bracewell *et al*. 2017; Feller *et al*. 2021; Beaudry *et al*. 2022; Katsumi *et al*. 2022). Environmental heterogeneity can cause sex-specific selection and fitness to differ between environments; these selective differences can drive the evolution of differences in sex determination, sex chromosomes and sexual dimorphism in populations in the contrasting environments (e.g., Oke *et al*. 2018). The role of sex-specific selection according to ecology is relatively unclear at this time but likely to be central to the understanding of sex determination and sex chromosome diversity within and between species and over time (Abbott *et al*. 2017; Furman *et al*. 2020).

In addition to the effects on sex, environmental variation between habitats drives adaptive divergence of populations through local adaptation by divergent natural selection and the accumulation of reproductive isolation (Barton and Hewitt 1989; Coyne and Orr 2004; Butlin 2010; Johannesson *et al*. 2020). A variety of traits can be involved in reproductive isolation-including behavioural, genetic, ecological, or morphological. These traits are frequently sexually antagonistic, so may play a role in both sex chromosome evolution and adaptive divergence (Qvarnström and Bailey 2009; Rabosky 2016). Behavioural and morphological cues that are involved in mate choice are a common example of this (e.g., colour in guppies; Wright *et al*. 2017). Differences in signal traits and preferences between populations can produce assortative mating, an important component of reproductive isolation. Reproductively isolating loci are often found on sex chromosomes (for example, Kitano *et al*. 2009; Smith *et al*. 2016; Bracewell *et al*. 2017), potentially due to their sexual antagonism, giving sex chromosomes an important role in adaptive divergence. The same selection pressures and loci may therefore drive both sex and population differentiation. Sex chromosomes are also known to contribute to divergence and speciation through a number of other processes, such as Haldane's rule and the large-X and faster-X effects (Charlesworth *et al*. 1987; Charlesworth and Charlesworth 2000; Coyne and Orr 2004; Presgraves 2008; Lasne *et al*. 2017).

1.3 Reproductive isolation through adaptive divergence and habitat choice, and the role of inversions

Reproductive isolation between diverging populations can be increased through association of loci for multiple isolating traits that is influenced by genomic architecture (Ravinet *et al*. 2017). Loci may be pleiotropic or be physically linked in the genome, through proximity and mechanisms that impede recombination (Foote 2018). The suppression of recombination between multiple locally adaptive and/or isolating traits prevents the break-up of

advantageous combinations of alleles at these loci by gene flow (Jackson *et al*. 2016). Processes that cause recombination suppression are therefore selected for, as individuals with these combinations will have greater fitness than any hybrid offspring with recombinant haplotypes following gene flow (Kirkpatrick and Barton 2006). Chromosomal inversions have been shown in multiple species to differ between diverging populations, highlighting their role in recombination suppression during the divergence process (Joron *et al*. 2011; Wang *et al*. 2013; Lee *et al*. 2017; Fuller *et al*. 2018; Akopyan *et al*. 2022). Furthermore, inversions have been evidenced on sex chromosomes in a variety of taxa (Lahn and Page 1999; Wang *et al*. 2012; Vicoso *et al*. 2013; Wang *et al*. 2014; Wright *et al*. 2014; Natri *et al*. 2019; Shearn *et al*. 2020) and so are a possible mechanism for impeding recombination during their evolution (Charlesworth 1991), although they may accumulate following recombination suppression by other means (Sun *et al*. 2017; Furman *et al*. 2020). The same inversions may therefore be involved in both sex and population divergence, especially given the multiple links between the two processes.

In general, the strength of divergent selection must be greater than the homogenising effect of gene flow between populations in order for divergence to proceed (Rice and Hostert 1993; Smadja and Butlin 2011). The presence of multiple components of reproductive isolation, and their association, strengthens the barrier to gene flow between populations (Coyne and Orr 2004; Ravinet *et al*. 2017). While gene flow is a common opponent to divergence, non-random dispersal though habitat choice often contributes to reproductive isolation (Edelaar *et al*. 2008). Habitat choice is considered to be movement that results in individuals spending more time in certain environments than expected if dispersal were random (Futuyma and Moreno 1988). This can affect the adaptive divergence process in multiple ways (Webster *et al*. 2012). Simulations show that, depending on the genetic mechanism, habitat choice can be a strong barrier to gene flow, promoting adaptive divergence (Berner and Thibert-Plante 2015). Locally adapted individuals will be selected against and show reduced fitness in dissimilar environments to their own (Nosil *et al*. 2005); therefore, factors such as habitat choice will be favoured if they result in individuals spending a greater proportion of time in their native environment- acting effectively as a form of reinforcement. Further, when individuals disperse in this manner, they are more likely to breed with other individuals from their own population, i.e. adapted to the same habitat, thereby reducing interbreeding and so gene flow between divergently adapted populations (Maynard Smith 1966; Rice 1984a; Camacho *et al*. 2020). Habitat choice can therefore be an important driver of assortative mating and reproductive isolation (Webster *et al*. 2012).

1.4 *Littorina saxatilis* as a system for the study of sex determination and adaptive divergence

In order to test such questions- on drivers and mechanisms of sex determination and adaptive divergence and their interface, a diverse set of model systems is required. The intertidal snail *Littorina saxatilis* already provides a valuable system for research into local adaptation and divergence with gene flow, and the genetic mechanisms underlying these (Johannesson *et al*. 2020). It is highly amenable to studies of this nature due to a number of factors. It occupies highly heterogeneous shores across its range, where distinct locally adapted ecotypes have repeatedly evolved in response to varied selection pressures across the shore (Johannesson

et al. 2010; Butlin *et al*. 2014). In Sweden, two predominant ecotypes exist that have been studied for many years (Johannesson *et al*. 2020). The 'Wave' ecotype inhabits rocky headlands and has adapted to withstand the action of waves; it is small and thin-shelled with a relatively large aperture, which maximises anchoring to the rock and enables sheltering in small crevices (Boulding and Van Alstyne 1993; Johannesson *et al*. 2010; Le Pennec *et al*. 2017). More sheltered bays offer boulder-field habitat where the prevalent selection pressure on *L. saxatilis* is predation by crabs. In response, the 'Crab' ecotype has evolved large, thick shells with small apertures that can resist predation (Boulding and Van Alstyne 1993; Johannesson *et al*. 2010; Boulding *et al*. 2017).

Sharp transitions between the two environments and a low lifetime dispersal create narrow hybrid zones (Janson 1983; Reid 1996; Erlandsson *et al*. 1998; Panova *et al*. 2006; Hollander *et al*. 2015), where ecotypes readily hybridise despite a low level of reproductive isolation (in the form of habitat choice and assortative mating in addition to local adaptation) (Janson 1983; Erlandsson *et al*. 1998; Rolán-Alvarez *et al*. 1999; Cruz *et al*. 2004; Hollander *et al*. 2005; Perini *et al*. 2020). Hybrid zones provide opportunities for investigating the association of environmental and genetic factors with population divergence to help identify important selection pressures (Barton and Hewitt 1985; Barton and Hewitt 1989; Schilthuizen and Lammers 2013). Genetic and phenotypic clines between *L. saxatilis* ecotypes are replicated across populations spanning the environmental transitions on the shore (Butlin *et al*. 2014; Hollander *et al*. 2015; Westram *et al*. 2018; Westram *et al*. 2021). More recently, multiple polymorphic chromosomal inversions have been identified that show clinal variation between the ecotypes and are associated with adaptive traits (Westram *et al*. 2018; Faria *et al*. 2019; Morales *et al*. 2019; Koch *et al*. 2021; Westram *et al*. 2021; Koch *et al*. 2022). Traits such as size and shape are both sexually dimorphic and divergent between ecotypes (Grahame *et al*. 2006; Johannesson *et al*. 2010; Butlin *et al*. 2014; Larsson *et al*. 2020); linkage group 12 (LG12) has recently been shown to influence some of these traits in addition to holding a strong quantitative trait locus (QTL) for sex (Koch *et al*. 2021; Koch *et al*. 2022). QTL for other adaptive traits and outlier SNPs are also located on LG12 (Morales *et al*. 2019; Koch *et al*. 2021; Westram *et al*. 2021; Koch *et al*. 2022). In combination, this makes *L. saxatilis* an ideal system for the study of the interaction between sex determination and adaptive divergence, and the role of chromosomal inversions in these processes.

1.5 Aims, chapter overview, and contributions

I aimed to uncover and characterise the sex determination system(s) in populations of Swedish *L. saxatilis*, including any population- or habitat-specific variation, through analysis of sex-genotype-ecotype associations and testing for sex-specific inversions. Further, I aimed to use experimental techniques to test for habitat-based divergent selection and habitat choice and their association with inversions, to help understand their roles in adaptive divergence of the ecotypes. Alongside this, sex-biased selection on any sex-linked inversions was also tested experimentally. In conjunction, these studies aimed to offer insight into the relationships between sex-determination systems, inversions, and ecotype divergence, and the selective pressures underlying them.

In Chapter Two, I test for the presence of a sex-determination system in one population of *L. saxatilis*, and for inversions on the linkage group of interest. I characterise the varying sex-inversion associations in both ecotypes across the transect and discuss potential scenarios of sex- and ecotype-specific patterns of selection that may have produced the variation observed. This chapter has been published (Hearn *et al*. 2022); this is published open access under a Creative Commons Attribution Licence and I retain copyright. The data analysed in this chapter were gathered by Westram *et al*. (2018). I conducted the analyses presented in this chapter except for the linkage mapping, which was conducted by Eva Koch for the F2 crosses (Koch *et al*. 2021) and Pragya Chaube for the Crab ecotype family (Westram *et al*. 2018). Sean Stankowski assisted with the read mapping pipeline for the diversity and divergence analysis. I wrote the paper, with input from all authors.

In Chapter Three, I extend this to include population-specific variation: I utilise replicate hybrid zones across three additional islands to test whether the same sex-determination system and inversions are present and gain understanding of the importance of sex and sex determination in adaptive divergence and vice versa. Comparisons of equivalent populations with slight variations in habitat and therefore selective pressures offer insight into the drivers of the above processes. The data analysed in this chapter were gathered by Westram *et al*. (2021) and I conducted all analyses.

In Chapter Four, I utilised a mark-recapture reciprocal transplant approach to test the role of habitat choice and divergent selection in local adaptation through examining differences in survival, phenotypes, movement, and inversion genotypes between ecotypes and habitats and over time. Genotyping of sex-linked inversions in this experiment provided additional opportunities to study sex-biased divergent selection and the role of sex-linked inversions in local adaptation. I designed the experiment with Kerstin Johannesson and Roger Butlin. Fieldwork and lab processing were carried out by Roger Butlin, Kerstin Johannesson, Andrea Cabrera and myself, with help from several others for the searches. SNP genotyping was carried out by LGC Genomics. I conducted all analyses.

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CHAPTER TWO
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Differing associations between sex determination and sex-linked inversions in two ecotypes of *Littorina saxatilis*

2.1 Abstract

Sexual antagonism is a common hypothesis for driving the evolution of sex chromosomes, whereby recombination suppression is favoured between sexually antagonistic loci and the sex-determining locus to maintain beneficial combinations of alleles. This results in the formation of a sex-determining region. Chromosomal inversions may contribute to recombination suppression but their precise role in sex chromosome evolution remains unclear. Because local adaptation is frequently facilitated through the suppression of recombination between adaptive loci by chromosomal inversions, there is potential for inversions that cover sex-determining regions to be involved in local adaptation as well, particularly if habitat variation creates environment-dependent sexual antagonism. With these processes in mind, we investigated sex-determination in a well-studied example of local adaptation within a species: the intertidal snail, *Littorina saxatilis.* Using SNP data from a Swedish hybrid zone, we find novel evidence for a female-heterogametic sex determination system that is restricted to one ecotype. Our results suggest that four putative chromosomal inversions, two previously described and two newly discovered, span the putative sex chromosome pair. We determine their differing associations with sex, which suggest distinct strata of differing ages. The same inversions are found in the second ecotype but do not show any sex association. The striking disparity in inversion-sex associations between ecotypes that are connected by gene flow across a habitat transition that is just a few metres wide indicates a difference in selective regime that has produced a distinct barrier to the spread of the newly discovered sex-determining region between ecotypes. Such sex chromosome-environment interactions have not previously been uncovered in *Littorina saxatilis* and are known in few other organisms. A combination of both sex-specific selection and divergent natural selection is required to explain these highly unusual patterns.

2.2 Introduction

Species with separate sexes experience evolutionary challenges because males and females are subject to different patterns of selection (Connallon 2015) and, therefore, fitness effects of some alleles differ between the sexes (Connallon and Clark 2014). The appearance of such sexually antagonistic alleles, followed by suppression of recombination to link sexually antagonistic loci and the sex-determining locus to avoid fitness cost, is a common hypothesis for driving the evolution of sex chromosomes (Fisher 1931; Rice 1987; Rice 1996; Wright *et al.* 2016) although alternative models are available (e.g. Lenormand & Roze 2022). Despite an increase in research into nascent sex chromosomes and interspecies comparisons, it remains challenging to test models for the drivers of sex chromosome evolution (Wright *et al.* 2016; Abbott *et al.* 2017; Vicoso 2019; Furman *et al*. 2020). For example, sex chromosomes are the most advantageous location in the genome for the emergence of sexually antagonistic

alleles, so it is unclear whether these loci drive sex chromosome evolution or accumulate after chromosome differentiation (Rice 1984; Charlesworth *et al.* 2005). Chromosomal inversions are one possible mechanism for impeding recombination in the heterogametic sex (Charlesworth 1991). Inversions on sex chromosomes have been observed in a number of taxa, including birds (Wang *et al.* 2014; Wright *et al.* 2014), primates (Lahn and Page 1999; Shearn *et al.* 2020), fish (Natri *et al.* 2019), snakes (Vicoso *et al.* 2013), and papaya (Wang *et al.* 2012). However, inversions can be a consequence of, rather than a mechanism for, recombination suppression (e.g. *Neurospora tetrasperma*; Sun *et al.* 2017). Lack of recombination due to other means removes selection for gene order, allowing structural variants such as inversions to accumulate (Furman *et al*. 2020). Species with young, emerging sex chromosomes are likely to be valuable systems for addressing such questions, because it is possible to make intraspecific comparisons where the genomic basis of sex is labile. Few studies have utilised this opportunity (Furman *et al.* 2020).

Sex chromosome evolution is usually assumed to occur in a homogenous environment. In reality, environments, populations and patterns of selection are heterogeneous in space and time. The potentially complex effects of this heterogeneity on sex chromosome evolution are important, but greatly understudied (Abbott *et al.* 2017). For example, divergent selection may drive frequency differences of inversion arrangements that suppress recombination between loci for locally adaptive traits and are therefore useful for local adaptation (Kirkpatrick and Barton 2006; Joron *et al.* 2011; Wang *et al.* 2013; Lee *et al.* 2017). However, it remains unknown if the same inversions are associated with sex chromosome evolution and local adaptation, and whether the two different processes interact.

There are clear similarities between the processes of adaptive divergence and sex chromosome evolution. In both cases, inversions are thought to maintain beneficial combinations of alleles at different loci (Charlesworth 2016; Huang and Rieseberg 2020). The two processes might interact if a driver of sex chromosome evolution, e.g. sexual antagonism, was environment-dependent and so drives differential evolution of sex chromosomes between populations (Bracewell *et al.* 2017; Wright *et al.* 2017; Lasne *et al.* 2018). Traits such as size (e.g., in *Littorina saxatilis;* Perini *et al.* 2020) or colour (e.g., in guppies; Wright *et al*. 2017) that are important in one sex for mate choice may also confer greater negative fitness effects in certain environments according to strength of selection pressures such as predation. For example, a young sex chromosome could influence gene flow with a connected population that does not experience sexual antagonism, or an inversion that captures locally adapted alleles may also capture the sex-determining locus and so inhibit the spread of a nascent sex chromosome into other environments.

With these processes in mind, we investigated sex determination in a well-studied example of local adaptation with gene flow, the intertidal snail *Littorina saxatilis* (Johannesson *et al.* 2020). The species is ovoviviparous, contributing to low lifetime dispersal, which has facilitated local adaptation over small spatial scales (Reid 1996). Two distinct ecotypes have adapted to differing rocky shore habitats (Johannesson *et al.* 2010; Butlin *et al.* 2014). In Sweden, the Crab ecotype inhabits boulder fields and has evolved to withstand crab predation. It has a larger, thicker, elongated shell with a relatively smaller aperture and is more wary in its behaviour (Figure 1) (Johannesson *et al.* 2010). The Wave ecotype is adapted to withstand wave action on rocky headlands via a smaller, thinner, globose shell that allows sheltering in small crevices (Figure 1) (Johannesson *et al.* 2010). Despite this ecological selection and

Figure 1. Image of the Crab-Wave ecotype transition sampled on Ängklåvebukten. The hybrid zone is the area where the boulder field and rocky cliff habitats meet. Inset are images of typical Crab and Wave individuals of *Littorina saxatilis.*

some degree of habitat and mate choice (Johannesson *et al.* 2010), the ecotypes readily hybridise (Panova *et al.* 2006; Hollander *et al.* 2015; Westram *et al.* 2018).

Genetic and phenotypic clines between the ecotypes are replicated at many locations across the species range (Grahame *et al.* 2006; Galindo *et al.* 2019; Westram *et al.* 2021). Multiple putative inversions have been identified in *L. saxatilis*, some of which show systematic frequency differences between the ecotypes and are associated with adaptive traits (Westram *et al.* 2018; Faria *et al*. 2019; Morales *et al.* 2019; Koch *et al.* 2021; Westram *et al.* 2021). *L. saxatilis* has separate sexes that are genetically determined (Fretter and Graham 1962). However, strongly heteromorphic sex chromosomes have not been observed, leaving the sex-determination mechanism unknown (García-Souto *et al.* 2018). Sexual dimorphism has been identified in reproductive anatomy (Fretter and Graham 1962) and traits such as size and shape (Larsson *et al.* 2020). Size-assortative mating creates sexual selection for a smaller male size (Perini *et al.* 2020). A recent study using crosses of Crab and Wave ecotypes of Swedish *L. saxatilis* found a strong QTL for sex on one linkage group (LG12) (Koch *et al.* 2021) but did not characterise the sex-determination system. Combined with the knowledge of multiple putative inversions (Faria *et al.* 2019), including two on LG12 (that showed frequency differences between ecotypes but were not tested for associations with sex), this makes *L. saxatilis* an ideal system to study the interaction between sex chromosome evolution and local adaptation within a species.

Here, we test for the presence of a sex-determining region in *L. saxatilis* through analysis of sex-specific patterns in SNP data from a transect of snails across a hybrid zone in Sweden. We find evidence for a female heterogametic sex chromosome system, but only in the part of the transect that is inhabited by the Crab ecotype. Almost the entire length of LG12 is spanned by four putative inversions, but they show varying levels of sex and ecotype differentiation.

They may represent distinct strata of a non-recombining region whose evolution has apparently been influenced by barriers to gene flow between ecotypes.

2.3 Methods

Sampling and genotyping

This study utilised a dataset previously published in Westram *et al.* (2018). Sampling and data generation methodology are described in brief here; for full details, see Westram *et al.* (2018).

Six hundred snails were sampled along a transect that crossed the Crab-Wave ecotype transition from boulder field to rocky cliff at Ängklåvebukten (Swedish west coast; 58°52'15.14"N 11°07'11.88"E; Figure 1). Snail positions were recorded in three dimensions. Positions were subsequently collapsed to a one-dimensional path to facilitate cline analysis. Size, shape and sex was determined for each snail. DNA was extracted from 373 sexually mature snails as described in Panova *et al.* (2016), before capture sequencing using 40,000 120-bp probes, randomly distributed across the genome. Read mapping to the reference genome (Westram *et al.* 2018), quality control, filtering and genotyping were conducted as described in Westram *et al.* (2018). The only difference in this study was during the generation of an additional VCF for LG12, with the exclusion of the --variants-only argument in the command bcftools (v1.11) call, and reducing minimum alleles required from 2 to 1 during VCF filtering. This resulted in an all-sites VCF including invariant sites and SNPs, covering 12,355kbp (on LG12) of the 1.35Gbp genome (all 17 LGs).

Data preparation

All analyses were performed using R (v4.0.0; R Core Team 2021) and the packages DPLYR (v1.0.5; Wickham *et al.* 2021) and GGPLOT2 (v3.3.0; Wickham 2016) unless otherwise stated. Only genotyped snails were used (205 females and 168 males). For some analyses the position of the snail on the transect, relative to the main environmental transition at 78m (Westram *et al.* 2018), was used to classify snails by ecotype: <68m Crab, 68-88m hybrid, >88m Wave. For analyses that required exclusion of hybrids, 64 male and 57 female hybrids were removed to leave a total of 252 snails: 90 Crab females, 62 Crab males, 58 Wave females and 42 Wave males. Greater numbers of females were likely due to a sampling bias toward larger individuals (Perini *et al.* 2020), rather than a sex-ratio bias in the population.

Detection of a sex-associated region

A QTL for sex (Koch *et al.* 2021) is located on linkage group 12 (LG12), and initial analyses for sex-associated SNPs (see below) yielded only SNPs on LG12. Therefore, of the set of SNPs produced from the capture sequencing, only SNPs located on contigs in the reference genome that mapped to LG12 were retained (linkage map from Westram *et al.* (2018)). Eight contigs contained SNPs with more than one assigned map position; for this small number of SNPs, the most common map position for the contig was used. This gave a total dataset of 8657 SNPs with map positions located on 713 contigs on LG12.

Genotype and allele frequencies were calculated for each SNP, separately for each sex and ecotype. The frequency of heterozygotes for each SNP was compared between the sexes. SNPs in sex-determining regions (linked to the sex-determining locus, potentially with

recombination suppression creating divergence between nascent Z and W chromosomes) are expected to diverge in genotype and allele frequencies between the sexes (Pucholt *et al.* 2017; Palmer *et al.* 2019). SNPs outside these regions are not expected to show significant differences between sexes. Deviations from this expectation were quantified by measuring residuals from the 1:1 relationship between proportions of heterozygotes in males and females (male heterozygosity minus female heterozygosity). These residuals were then plotted on the linkage map to indicate the position of the sex-determining region.

Sex-specific recombination maps were examined to detect any difference between the sexes. A sex-determining region is expected to show recombination suppression in the heterogametic sex; recombination is also suppressed in individuals of either sex that are heterozygous for an inversion. Maps were available from a Crab x Crab cross (Westram *et al.* 2018) and from Crab x Wave crosses (Koch *et al.* 2021), both using individuals from the population sampled in our transect. The Crab x Wave maps were produced from several families whose parents were Crab x Wave hybrids, while the Crab x Crab map was a product of a single pair of parents. Unless otherwise stated, the sex-averaged Crab x Crab map was used to position contigs.

An ecotype limited, sex-associated region

When heterozygote proportions were compared between the sexes, only LG12 showed strong sex differences and as such only LG12 was retained for further analysis. However, separate examination of heterozygosity on LG12 in each ecotype revealed that sex differences were limited to the Crab ecotype (see below). As a result, comparisons of heterozygosity were repeated for all other linkage groups with only Wave individuals to test for a sex-associated linkage group in this ecotype that may have been masked when both ecotypes were analysed together. 255,114 SNPs with map positions on 11,155 contigs across the 17 linkage groups were used. The distribution across the 17 linkage groups of the 1% of SNPs with the most negative residuals was used to test for a sex-associated linkage group.

Inversion detection using linkage disequilibrium and principal component analyses

A similar methodology to the one used in Faria *et al.* (2019) to detect putative chromosomal inversions in *L. saxatilis* was implemented here for LG12. These analyses exploit the expectation of high linkage disequilibrium (LD) for loci in polymorphic inversions, compared to surrounding regions. Since inversions across the sex-determining region may differ between males and females, LD analysis of both sexes together may mask detection of groups of SNPs that are in high LD in one sex only. Therefore, only females were used for the LD analysis (male data were included in the next step of cluster investigation). All females from across the transect were included.

Briefly, the package GENETICS (v1.3.8.1.2; Warnes *et al.* 2019) was used to generate a matrix of pairwise LD (r^2) values for all SNPs. This LD matrix was then used with the package LDNA (v0.6.4; Kemppainen *et al.* 2015) to identify 'outlier clusters' of SNPs that showed higher LD than the rest of the linkage group. The package allows variation in two parameters that affect the detection of clusters: $|E|_{min}$ and φ. These were manipulated, similarly to in Faria *et al.* (2019), to produce a set of outlier clusters of interest (see Supplementary Methods for details of parameter combinations and criteria).

To investigate the clusters of SNPs in high LD identified by LDNA, principal component analysis (PCA) was utilised. When high LD clusters are generated by inversions, SNPs are expected to be clustered in one region of a linkage group and a PCA of that region groups individuals by inversion genotype (two homozygote groups and one heterozygote group, if two arrangements are present). Other causes of high LD clusters are unlikely to share these properties (see Kemppainen *et al.* (2015) and Faria *et al.* (2019) for a more detailed discussion). Therefore, we examined the position of SNPs in each cluster on the LG12 genetic map and performed PCA on all SNPs (not just those in the LD cluster) in each cluster region. PCA was carried out using R packages HMISC (v4.4.0; Harrell Jr 2020) and ADEGENET (v2.1.3; Jombart 2008; Jombart and Ahmed 2011) on the male and female data together.

Cline fitting

Cline analysis was conducted for putative inversions identified on LG12 to examine changes in frequency across the hybrid zone from Crab to Wave and any differences between the sexes. Clines were fitted for the putative inversion arrangement that was more frequent in Crab than Wave in females (or in males when the frequency in females did not vary). This arrangement was labelled R (reference arrangement), and the other A (alternate).

Clines were fitted to putative inversion genotypes across the transect using a simple sigmoid model, following the formulation from Derryberry *et al.* (2014), and using the mle2 function in the R package BBMLE (v1.0.23.1; Bolker and R Development Core Team 2020). Five models were fitted: a null model (no change in arrangement frequency), the full model (separate parameters for male and female centre, width, Crab and Wave frequencies), and three constrained models to test parameter differences between males and females: 'combined' (all parameters equal between sexes), 'constrained' (only centre and width equal between sexes), and 'Wave-constrained' (centre, width and Wave frequency equal between sexes). No Crab-constrained model was included as sex differences in arrangement frequency are expected in Crab for sex-linked inversions. We also considered the possibility of no cline in one sex and a cline in the other. The AIC of each model was used to test which best fitted the data and therefore whether cline parameters differed between the sexes. For illustration, arrangement frequencies for each putative inversion were calculated along the transect, for each sex, in overlapping sliding windows of 25 snails shifting by 5 snails.

Divergence and diversity estimates

Genetic diversity (π) and divergence (d_{XY}) were calculated for putative inversion genotypes for an insight into the age and sequence of evolution of the inversions. An all-sites VCF was used for calculation of these statistics since this is known to reduce bias in the estimates. Calculations of π and d_{XY} were carried out separately for each putative inversion using custom scripts from Martin (2020). Individuals were split into three groups according to putative inversion genotype (heterozygotes and the two homozygote groups) and also by sex and ecotype (giving up to 12 'populations' when the three genotypes were present in both sexes and ecotypes). π was calculated within each of these groups and d_{XY} between each pair of groups. Since the reference genome for *L. saxatilis* is not contiguous, statistics were calculated for each contig by setting a non-overlapping window size of 2000bp; a small number of large contigs were split into two or three windows using this window size.

d_{XY} between inversion arrangements was calculated using the π values for inversion genotypes using the following equation:

$$d_{XY}\ between\ A\ and\ R = 2(\pi_{RA} - \frac{\pi_{RR}}{4} - \frac{\pi_{AA}}{4})$$

where π_{RA}, π_{RR} and π_{AA} are the nucleotide diversities for the heterokaryotypes and two groups of homokaryotypes, respectively. This equation makes allowance for the fact that half of the comparisons in the heterozygotes are between arrangements and the other half are within one arrangement or the other.

To test the effect of genotype, sex, and ecotype on nucleotide diversity in putative inversion arrangements, mixed models were fitted to the π values calculated for groups of individuals of each combination of these variables, separately for each putative inversion, using lme4 (v1.1.27; Bates *et al.* 2015) and MuMIn (v1.43.17; Barton 2009). See Supplementary Methods for details.

Inversion genotype-genotype and genotype-sex associations

Associations between genotypes at different putative inversions, and between each putative inversion and sex, were assessed using chi-square contingency tests in the packages ZOO (v1.8.8; Zeileis and Grothendieck 2005) and TIDYQUANT (v1.0.3; Dancho 2021). This was carried out separately for each ecotype. Squared correlation coefficients were used to measure the strength of association.

2.4 Results & Discussion

Our data suggest a female-heterogametic (ZW) sex determination system in the Crab ecotype of *Littorina saxatilis* at our study site in Sweden. The sex chromosome pair contains four regions of suppressed recombination, consistent with putative chromosomal inversions, some of which behave like strata on the sex-specific (W) chromosome. However, these putative inversions are not associated with sex in the Wave ecotype at the same site and the sex determination system for the Wave ecotype remains uncertain. Below, we present the evidence that leads to these novel conclusions and then consider scenarios that might have led to the different patterns between the ecotypes.

Female-heterogametic sex determination in the Crab ecotype

Association of genotypes with sex can be one of the first indicators of the evolution of a young sex-determining region (Pucholt *et al.* 2017; Palmer *et al.* 2019). In our data, while most LG12 SNPs followed the neutral expectation of equal proportions of heterozygotes in each sex, a group of SNPs departed strongly from this expectation (Figure 2a). In these deviating SNPs, heterozygosity was skewed towards females but few were heterozygous in all females suggesting that they are linked to, rather than at, a sex-determining locus. SNPs showed varying strengths of association with sex as reflected by the continuous distributions of heterozygosity (Figure 2a) and residuals (Figure 2c). There was a striking difference between the two ecotypes: Crab snails showed many sex-associated SNPs, with some close to perfect association (all females heterozygous, all males homozygous) but there was no such

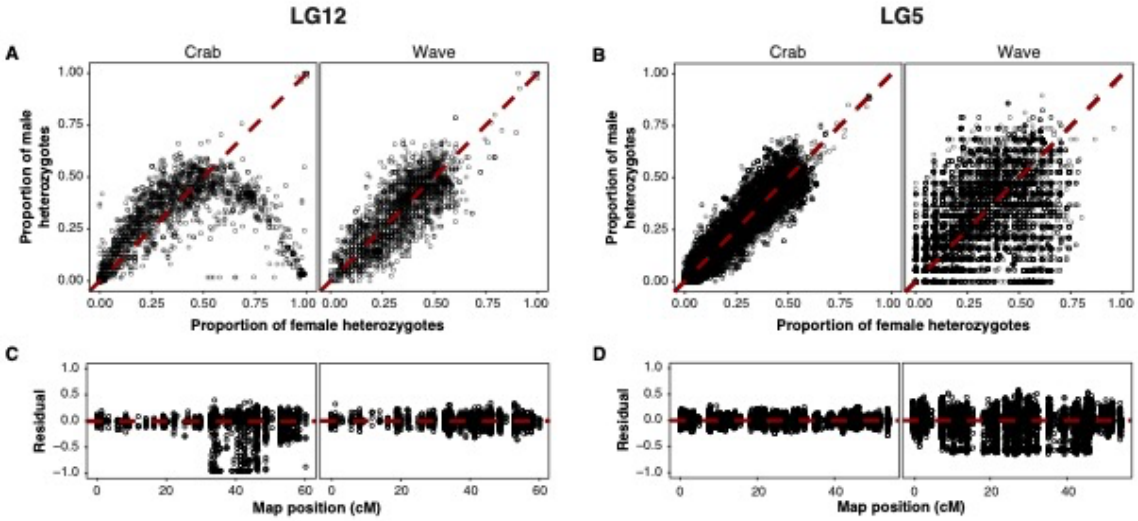

Figure 2. The proportions of each sex that are heterozygous at SNPs on **A)** LG12 and **B)** LG5 in the two ecotypes. SNPs with a greater difference in heterozygosity between the sexes are further from the 1:1 line (neutral expectation of equal heterozygote proportions between the sexes). The distribution of sex-associated SNPs on **C)** LG12 and **D)** LG5 along their respective genetic maps. Residuals quantify the deviation of SNPs from neutral expectation, calculated as female heterozygosity - male heterozygosity.

association in the Wave individuals. These results indicate a ZW sex-determining system in the Crab ecotype but provide no evidence concerning the Wave sex determination system.

Sex-associated loci are expected when recombination has ceased in a region of the chromosome surrounding the sex-determining locus, so that loci in this region build up LD with the sex-determining locus (Abbott *et al.* 2017). Therefore, we checked how the sex-associated SNPs were distributed along the genetic map for LG12, with the expectation that sex-associated SNPs are clustered. The pattern described is for the Crab ecotype as sex-associated SNPs were found only in this group. The first half of the linkage group up to 32.8cM did not hold any sex-associated SNPs (Figure 2c). Nearly all strongly sex-associated (large residual) SNPs clustered in a central area between 33.0cM and 48.7cM, with medium-residual (marginally sex-associated) SNPs also distributed up to the end of the linkage group from 48.7cM to 60.2cM. As theory predicts that the most strongly sex-associated loci cluster around the sex-determining locus, this suggests that a sex-determining locus in *L. saxatilis* is located in the region from 33.0 to 48.7cM (LGC12.2 and LGC12.3; see below). Indeed, a strong QTL for sex (LOD=26, P<0.001) in *L. saxatilis* has recently been identified on LG12 (Koch *et al.* 2021) and is located in the central region of sex-associated SNPs. Thus, our data support the presence of a sex-determining region on LG12 and show that it is a female-heterogametic system, but only in the Crab ecotype.

Sex-determination in the Wave ecotype

All evidence for a female-heterogametic sex determination system was found in the Crab ecotype only, leaving the mechanism for sex-determination in the Wave ecotype unknown.

With such close proximity to the Crab ecotype, there is likely to be some genetic component of sex-determination in Wave; this may or may not involve the same sex-determining locus as in Crab. Any weaker patterns in Wave may have been masked by the strong Crab pattern. Therefore, the comparison of heterozygosity between sexes was repeated for all linkage groups with Crab and Wave individuals separated. Results were more variable in Wave, probably due to the lower sample sizes of Wave males and females, and displacement of clines into the Wave habitat (Westram *et al.* 2018) (Supplementary Figure 1). One linkage group, LG5, showed a likely signal (Supplementary Figure 1; Figure 2b) with some female-bias in heterozygosity, although much weaker than that seen on LG12 in Crab. About 60% of the most sex-associated SNPs (1% most negative residuals) were located on LG5 while no other linkage group held more than 6% of these SNPs (Supplementary Figure 1). SNPs with strongly negative residuals (female heterozygosity > male heterozygosity) were spread across much of LG5 (Figure 2d). One possible explanation for this pattern is a young ZW system, with less differentiation than for LG12 in Crab. In this study, we focus on the LG12 sex-determination system; future analysis is needed to determine any potential role of LG5 in Wave.

Putative inversions on LG12

Inversions are often found on sex chromosomes. They are hypothesised to be a key mechanism in the suppression of recombination during the evolution of sex chromosomes (Lahn and Page 1999; Wang *et al.* 2012; Natri *et al.* 2019), but they may evolve later following recombination suppression by other means. Whether a cause or consequence, inversions are expected in sex-determining regions.

Therefore, linkage disequilibrium (LD) and principal component analyses (PCA) were carried out to test for the presence of sex-specific inversions on LG12 that cover the region of sex-associated SNPs. Five outlier clusters of SNPs were identified, using LDNA for females, as regions of interest for downstream analysis (see Supplementary Results; Figure 3a; Supplementary Table 1). SNPs in each of the five clusters were distributed in distinct regions of LG12 (Figure 3a-b). Two clusters covering the first and last parts of LG12 match the positions of the inversions LGC12.1 and LGC12.2, respectively, from Faria *et al.* (2019). The other three clusters overlap and cover the central region of LG12 between the two described inversions (Figure 3a-b), suggesting previously undiscovered putative inversions that span the central region of LG12.

PCA was carried out on the three regions of LG12, both separately for males and females and with the sexes together. Six distinct clusters were present in the PCA of the central region, a pattern consistent with two neighbouring inversions in LD with one another. This was corroborated by examining genotypes of individuals across LG12 (Figure 3b; Supplementary Figure 2a-b Supplementary Figure 3; see Supplementary Methods and Results for a detailed explanation). This central region was therefore split into two according to the SNP and genotype distributions, and PCAs of these subregions each gave three distinct groups along PC1 with either no or very rare intermediate individuals (Figure 3c; Supplementary Figure 3). The overlapping clusters observed in LDNA (Figure 3a) were likely due to linkage disequilibrium between these two putative inversions. For the first and last region, separate PCAs of females and males (Supplementary Figure 3) revealed the expected three distinct groups, consistent with polymorphism of LGC12.1 and LGC12.2 (as detected by Faria *et al.* 2019) in both sexes. For each of the four putative inversion regions, snails from all locations

across the transect were present within the same three clusters indicating that the arrangements are shared between ecotypes. PCA using both sexes together for each region showed that males and females also fell into the same three distinct groups (one group is very small for region 43.8-48.7cM, where one putative homozygote is rare) (Figure 3c), i.e. sexes also share arrangements of the putative inversions. Arrangement frequencies are examined in the next section.

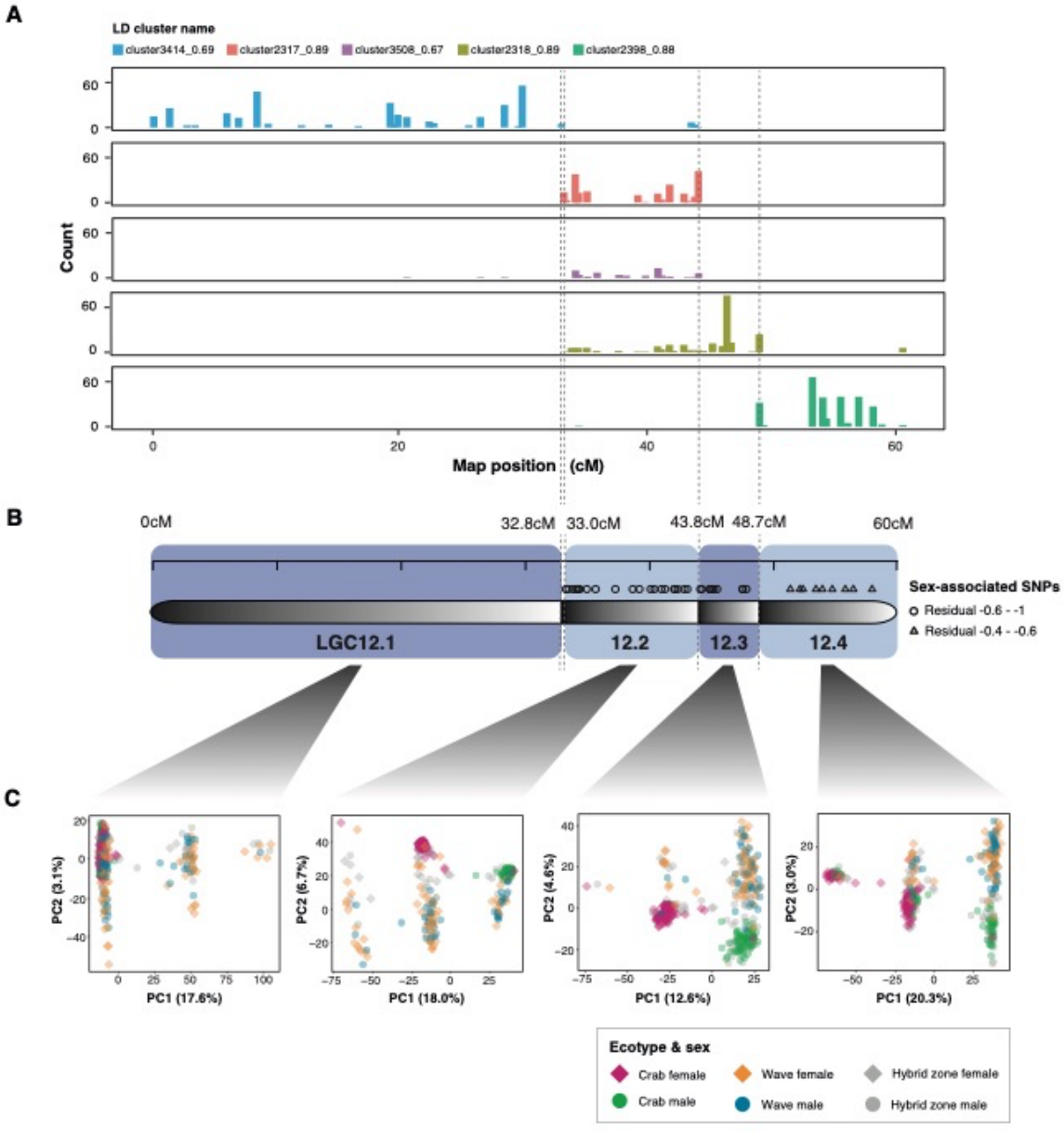

Figure 3. A) The distribution of SNPs along LG12 in each of the five LD clusters of interest, and how these correspond to **B)** four putative inversion regions on LG12 (illustrated by black-white gradients) and the distribution of sex-associated SNPS from Fig.1. **C)** PC1 vs PC2 from PCAs (scaled and centred) of SNPs in the four regions of LG12 covered by the LD clusters of interest. PCAs were carried out for all individuals (of both sexes and ecotypes) together.

The LD and principal component analyses supported the presence of four putative polymorphic inversions on LG12 (Figure 3b). From this point on, the putative inversions will be referred to simply as inversions, for brevity, and named LGC12.1 (the same as LGC12.1 from Faria *et al.* (2019)), LGC12.2, LGC12.3 and LGC12.4 to maintain the inversion naming system used in Faria *et al.* (2019). LGC12.2 of Faria *et al.* (2019) is renamed to LGC12.4 so that names are sequential along LG12.

Recombination is expected to be suppressed in individuals heterozygous for inversion arrangements and, therefore, genetic maps can help to confirm the presence of inversions. Maps for each sex from a Crab x Crab family (Westram *et al.* 2018) and a series of Crab x Wave families (Koch *et al.* 2021) further supported the presence of inversions in the genomic locations described (Figure 4). In the Crab x Crab map, both parents showed normal recombination in the first part of LG12, while recombination was absent in the female parent in the second half of the linkage group where sex-associated SNPs are found. This is consistent with the female parent being heterozygous for inversions LGC12.2, LGC12.3 and LGC12.4. In the Crab x Wave families, each parent showed a different pattern of recombination, consistent with different combinations of heterozygous inversions in these

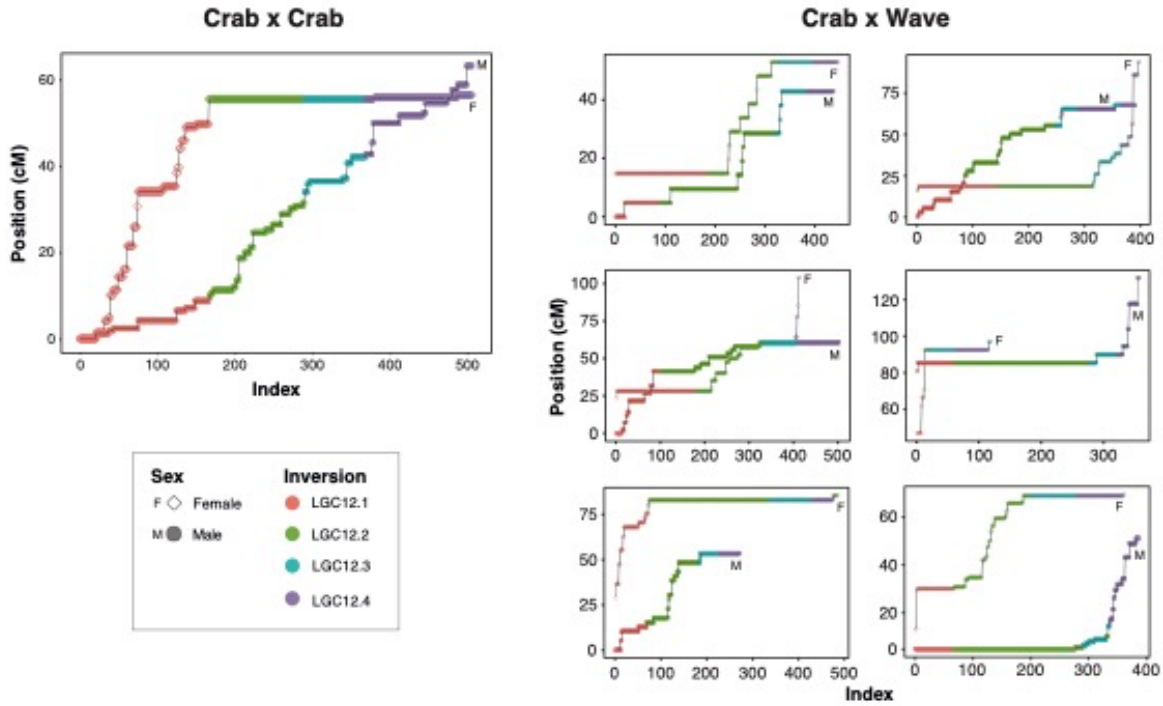

Figure 4. Male- and female-specific genetic maps of LG12 from a Crab x Crab family, and six Crab x Wave F2 families. For the Crab x Crab map, only markers informative in both sexes were used. In the Crab x Wave maps, markers informative in females only were used for the female map and markers informative in males only were used for the male map. In all panels, markers were numbered in order according to their position on LG12 (Index). Markers were coloured by putative inversion region, with assignment based on their positions relative to the outermost map positions of markers confidently assigned to each inversion. Markers that could not be assigned to an inversion were removed. Horizontal lines (i.e. no change in map position between successive markers) indicate an absence of recombination.

Table 1. Correlation (r^2) and significance of association of genotypes at pairs of inversions for males and females of the Wave ecotype. Correlation and significance of association of inversion genotype with sex is also given in the final column for the Crab and Wave ecotypes.

	LGC12.2		**LGC12.3**		**LGC12.4**		**Sex**	
	F	**M**	**F**	**M**	**F**	**M**	**C**	**W**
LGC12.1	**0.3969** (0.000133)	**0.5929** (4.82×10^{-5})	0.0064 (0.784)	0.0100 (0.352)	0.0100 (0.736)	0.0004 (0.499)	*N/A*	0.0036 (0.420)
LGC12.2			**0.1936** (0.0447)	0.0784 (0.387)	0.1225 (0.176)	0.0081 (0.745)	**0.9025** (4.24×10^{-30})	**0.0625** (0.0428)
LGC12.3					**0.7921** (2.30×10^{-10})	**0.462427** (0.0004)	**0.9025** (4.24×10^{-30})	0.0289 (0.220)
LGC12.4							**0.2116** (1.33×10^{-10})	0.0169 (0.393)

Abbreviations: F=female; M=male; C=Crab; W=Wave
Correlations with a significance of $p<0.05$ were highlighted in bold.

hybrid individuals. In each case, blocks of low recombination corresponded to one or more of the four inversions (Figure 4). These maps support the interpretation that Wave males can have any genotype for any of the four putative inversions, unlike Crab males which are nearly always homozygous for LGC12.2 and LGC12.3.

The fragmented *L. saxatilis* genome assembly and the capture sequencing approach used here preclude formal confirmation that regions of suppressed recombination are caused by inversions. However, other possible mechanisms of recombination suppression, in sex chromosome evolution and otherwise, such as transposable elements, heterochromatinization, methylation and epigenetic effects (Ironside 2010; Furman *et al.* 2020) are unlikely to produce the specific patterns we observe in this study (namely, the clustering of high LD SNPs in specific regions, the identification of three genetically distinct clusters of individuals by PCA, and the genotype-specific recombination suppression in experimental crosses) (Kemppainen *et al.* 2015; Faria *et al.* 2019).

Ecotype differences in sex-inversion associations

Associations among genotypes at putative inversions, and between putative inversions and sex, were quantified in both ecotypes. Inversions that are involved in sex chromosome evolution are expected either to contain the sex-determining locus or be in LD with it. Therefore, we predicted that LGC12.2, LGC12.3 and LGC12.4 would show significant association with sex and with each other in the Crab ecotype, but not the Wave ecotype. In the Crab ecotype, inadequate polymorphism meant associations among inversions could not be calculated: all individuals were fixed for one arrangement at LGC12.1, almost all females

were heterozygous at LGC12.2 and LGC12.3, and almost all males were homozygous for one arrangement at LGC12.2 and LGC12.3 (Figure 5). In the Wave ecotype, correlations between inversions were generally low and seven of the twelve pairwise comparisons were non-significant (Table 1). Significant correlations were present between LGC12.1 and LGC12.2 and between LGC12.3 and LGC12.4 in both sexes in Wave (Table 1). The following relationships fulfilled our predictions (Figure 5; Table 1): within Crab, LGC12.2 and LGC12.3 were significantly correlated with sex, LGC12.4 was less strongly correlated although the relationship was still highly significant, and in Wave correlations with sex were weak, although the order between them was the same (i.e. LGC12.2 showed the strongest, but only marginally significant, association; Table 1). A small number of Crab-like individuals present in the Wave habitat may have influenced these correlations (consistent with genome-wide clinal patterns seen in Westram *et al.* 2018).

Differences in arrangement frequency along the transect were quantified for males and females as a proxy for divergent selection on the arrangements between the ecotypes and to test how this differed between the sexes. If a difference in sex determination system between ecotypes is maintained by selection despite gene flow, inversions associated with sex (LGC12.2 and LGC12.3) will show clines in frequency between environments that differ between the sexes. Inversions not associated with sex (LGC12.1) may show clines, as previously shown in Faria *et al.* (2019), but these are not expected to differ between the sexes. The expectation for LGC12.4 is equivocal because of its partial association with sex.

Clines in arrangement frequency between ecotypes were detectable for all inversions for one or both sexes, indicating a role of divergent selection (Figure 6; Supplementary Tables 2-3). No inversion showed a sex difference in cline centre or width, and all fitted cline centres were close to the mean position of non-neutral clines from throughout the genome reported by Westram *et al.* (2018); the same environmental transition is likely to be driving selection on LG12 as the rest of the genome. As predicted, males and females showed little difference in arrangement frequency in either ecotype in LGC12.1, but arrangement frequencies differed between males and females in the Crab ecotype for the other three inversions. In addition, a

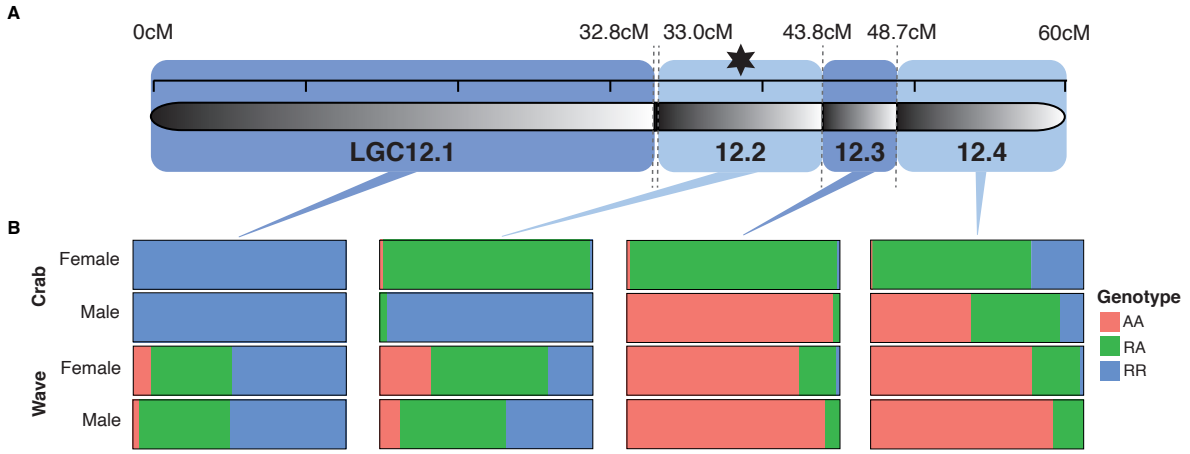

Figure 5. The location of the four putative inversions on LG12 and the proportions of inversion genotypes for each sex and ecotype group. Arrangements are labelled R (reference: the arrangement more frequent in Crab than Wave in females) and A (alternate); thus RR and AA are the two homozygote groups and RA is the heterozygote group. Black star indicates the approximate position of the QTL for sex (Koch *et al.* 2021).

small sex difference in arrangement frequencies may be present in the Wave ecotype for LGC12.2 (the 'Wave-constrained' model was marginally worse than the 'constrained' cline model). A clear shift in genotype frequencies for LGC12.2 was visible in females from Crab to Wave, from all heterozygotes to approximate Hardy-Weinberg proportions, despite there being no cline in arrangement frequency (Figure 6b).

Sex differences in SNP heterozygosity and putative inversion genotypes were found only in the Crab ecotype, while no sex differences could be seen among Wave individuals. Transitions in arrangement and genotype frequencies occurred over a short distance (0-23 metres; Supplementary Table 2). This indicates strong differential selection on the sex-determining region (LGC12.2 and LGC12.3) since ecotypes are connected by gene flow across the hybrid zone (Westram *et al.* 2018). Our PCA analysis confirmed that the three inversions (regions with suppressed recombination in heterozygotes) that are sex-associated in Crab were also present in Wave. However, as suggested by the lack of sex-association in Wave in the heterozygosity analyses, there is no evidence that the Wave ecotype follows the same sex determining system as we have found in Crab. The QTL for sex in the Crab x Wave F2 crosses (Koch *et al.* 2021) was produced by alleles derived from the Crab parents; Crab females and Wave males were used as parents for the crosses so any female-specific sex-determining alleles would be derived from the Crab ecotype.

Both arrangements of LGC12.2, the primary sex-determining region in Crab, were present in both sexes at an intermediate frequency in Wave. Wave females showed all three putative inversion genotypes in approximately Hardy-Weinberg proportions (Figure 5). Wave males similarly showed all three putative inversion genotypes, with a slightly higher frequency of 0.7 of the R arrangement (defined as the one more frequent in Crab than Wave in females, or in males if female frequency doesn't change). If the sex-determining locus is the same in Crab and Wave, for the three putative inversion genotypes to be present in both sexes in Wave, haplotypes of both the arrangements must exist with each of the alleles at the sex-determining locus to remove the sex-inversion association. In contrast, in Crab the female-specific allele at the sex-determining locus must be present on the A background only. This may be a shared haplotype with the Wave ecotype. Similarly, the other (male) allele at the sex-determining locus on the R arrangement in Crab may be shared across the transect into Wave. The lack of elevated divergence between Crab and Wave for any arrangement in either sex supports this idea (Supplementary Figure 4b).

The R arrangement of LGC12.3 was present at only a low frequency in the Wave ecotype (around 0.1), with the majority of Wave individuals of both sexes being homozygous for the A arrangement. In males, the R arrangement was present only near the hybrid zone, while Crab females were heterozygous. The presence of the R arrangement predominantly in Crab females suggests the origin of the R arrangement in this group and its failure to spread into the Wave habitat. This is consistent with the evolution of LGC12.3 as a second stratum of a female heterogametic sex chromosome in Crab. However, the presence of male heterozygotes and RR homozygotes of both sexes in the hybrid zone would indicate that rare recombination events occur in hybrids: If the putative inversions arose sequentially (as proposed below), recombination must have occurred between the close breakpoints of LGC12.2 and LGC12.3 to associate the R arrangement of LGC12.3 with an arrangement lacking the female sex-determining allele on LGC12.2. Individuals with these genotypes are

limited to the hybrid zone, however, implying that the genotypes are not fit enough to spread into either the Crab or Wave environment.

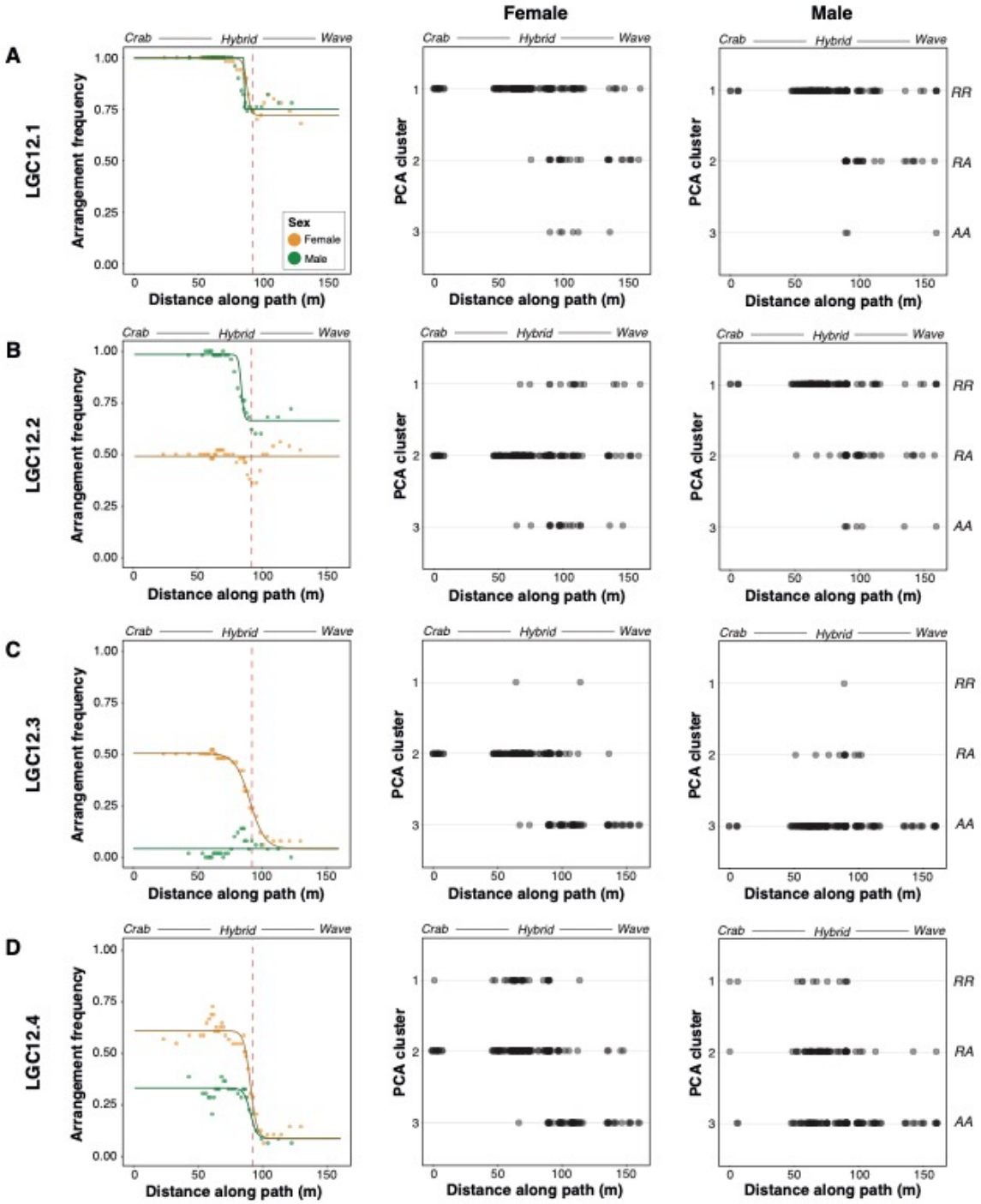

Figure 6. *Left-hand panels:* Frequency of R arrangement in windows of snails across the transect, and best fitting cline models of R arrangement frequency, in males and females for **A)** LGC12.1, **B)** LGC12.2, **C)** LGC12.3 and **D)** LGC12.4. Red dashed line shows the mean cline centre for non-neutral SNPs (91.8m) from Westram *et al.* (2018). Labels above each panel show the direction of transect (Crab - Hybrid zone - Wave); these labels are illustrative only because phenotypic and genetic clines vary in width and position. The best fitting cline models for each inversion (and for each sex

separately where the best fitting model differed between sexes) were as follows: LGC12.1 – full model; LGC12.2 females – null model, males – full model; LGC12.3 - Wave-constrained; and LGC12.4 - Wave-constrained. *Centre and right-hand panels*: Distribution along the transect of individuals in each PC1 cluster for **A)** LGC12.1, **B)** LGC12.2, **C)** LGC12.3 and **D)** LGC12.4 for females (*centre*) and males (*right*). PCA cluster 1 corresponds to R arrangement homozygotes; inversion genotypes (RR, RA, AA) are noted on the right-hand side for ease.

Sex differences in genotype and arrangement frequencies in both ecotypes were less distinct for LGC12.4. Similar to LGC12.3, the R arrangement was present at a low frequency in both sexes in the Wave ecotype and R homozygotes were rarely seen away from the hybrid zone. However, the strong genotypic differences between the sexes in LGC12.2 and LGC12.3 in Crab were not present for LGC12.4. The R arrangement differed in frequency slightly between males and females (0.3 and 0.6, respectively), but all three genotypes were seen in both sexes. Associations between LGC12.4 and the other inversions were generally rather low, suggesting one of two things: Either, this inversion did not evolve for reasons relating to sex and is just in LD with LGC12.3 due to their shared or close breakpoints, resulting in small sex differences in frequency. Or, any sex-specific benefits of the association of an LGC12.4 arrangement with sex (and therefore with the other inversions) are only just emerging, so the beneficial combination of arrangements among inversions has not yet spread. For example, if sexual antagonism was playing a role in this system, this could occur with a recent change so that a locus in LGC12.4 becomes sexually antagonistic, creating selection for association of a particular haplotype of a pre-existing inversion with sex.

Diversity and divergence of putative inversion arrangements

Genetic diversity (π) for each arrangement can be compared for an insight into which inversion arrangement is derived. Young inversions are expected to show low diversity in the derived arrangement compared to the ancestral, while the derived arrangement of older inversions is expected to have accumulated diversity over time, reducing the difference between the two arrangements (Andolfatto *et al.* 2001; White *et al.* 2009). At the same time, divergence (d_{XY}) between arrangements is expected to increase as they age.

The arrangement with lower π was identified through comparison of homokaryotypes for each arrangement. In the case of LGC12.3, where one homokaryotype was extremely rare, the heterokaryotype showed lower π than the abundant homokaryotype, implying a lower π in the rare than abundant homokaryotype. The R arrangement had lower π, and was inferred to be derived, for LGC12.3 and LGC12.4, while the A arrangement had lower π for LGC12.1 and LGC12.2 (Figure 7). Models confirmed that genotype significantly affected π for each of the four inversions (Supplementary Tables 5-6).

For the three inversions in the sex-associated chromosomal region, d_{XY} between arrangements was similar with a possible slight elevation in LGC12.4 (Figure 7). LGC12.2 showed the smallest difference in π between the two homokaryotypes, although estimates were again relatively similar between inversions (Figure 7). These d_{XY} and π estimates suggest that LGC12.2 may be the oldest of the sex-associated putative inversions. Estimates of d_{XY} did not reveal any marked differences between sexes or ecotypes for any inversion

(Supplementary Figure 4b), suggesting that no arrangement is diverging more rapidly than the others between sexes or ecotypes. However, there were differences in π between ecotypes and sexes for the sex-associated inversions (Supplementary Tables 5, 6), although estimates were generally much smaller than the genotype effect. The Crab ecotype showed reduced π compared to Wave in models for all three sex-associated inversions. Females showed reduced π compared to males in LGC12.3 and LGC12.4, but showed slightly higher π than males in LGC12.2. Significant effects of ecotype and sex on π are likely connected to the differing frequencies of arrangements among sexes and ecotypes, but may imply that there certain haplotypes of an arrangement are not shared between groups. Such differences can also be seen in the PCAs, where ecotypes are partly differentiated within each PC1 cluster (Figure 3c).

Sex chromosome strata in the Crab ecotype

The association of inversions with regions of sex-associated SNPs aligns with theory on sex chromosome strata. The putatively derived arrangements of LGC12.2 and LGC12.3 are restricted to the heterozygous females in Crab while males only exhibit the ancestral arrangement. These genotypes are expected if inversions are selected for recombination suppression in the heterogametic sex. The oldest stratum is expected to contain the sex-determining locus and LGC12.2, likely to be the oldest inversion on the basis of diversity and divergence, contains the sex QTL. Diversity estimates are less clear in distinguishing the age of LGC12.3 and LGC12.4. A greater difference in diversity between arrangements is visible in LGC12.3 but the comparison is unreliable due to the low derived arrangement frequency. The strong association of LGC12.3 with sex is a better indicator that it is older than LGC12.4 and evolved second. LGC12.4 shows much smaller sex differences in arrangement and SNP genotype frequencies, suggesting it may be the youngest stratum that has not yet spread throughout the population. However, this pattern may also be produced by differing amounts of recombination between putative inversions. More recombination between LGC12.3 and LGC12.4 than between LGC12.2 and LGC12.3 would result in a weaker association of

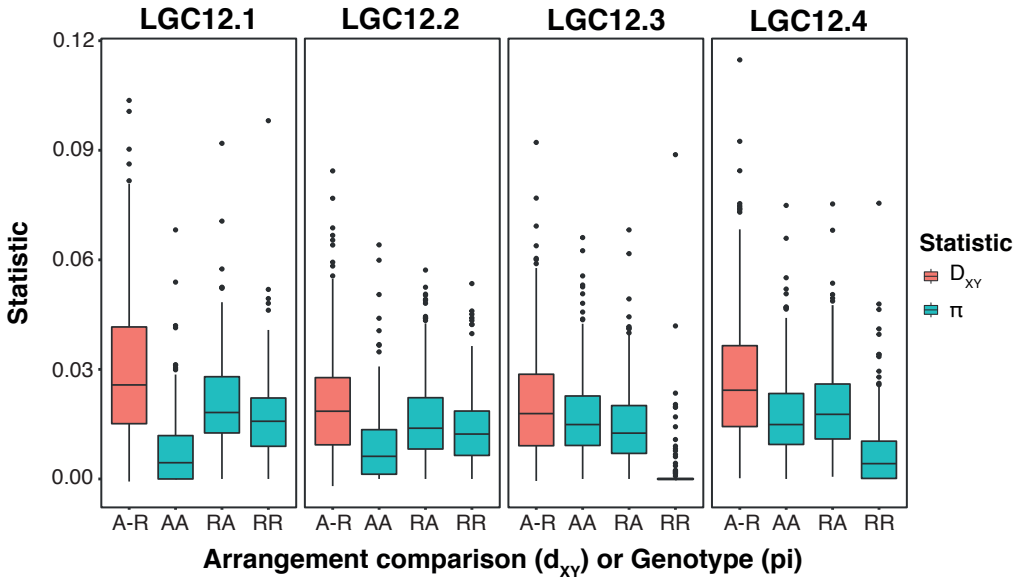

Figure 7. π per contig of each inversion genotype and d_{XY} between inversion arrangements for the four inversions.

inversion genotype with sex for LGC12.4. Conversely, LGC12.4 may only show sex-association due to chance build-up of LD between itself and LGC12.3 opposed only by low recombination. Although recombination was not observed in the region of sex-linked putative inversions in the Crab x Crab recombination map, the size of the family used means that a map distance of around 1cM between LGC12.3 and LGC12.4 remains plausible. Neither the genetic maps nor the genome assembly currently available make it possible to be certain of the relative positions of breakpoints for the four putative inversions.

Scenarios for sex- and ecotype-specific patterns of selection

Here, we speculate about possible evolutionary histories that may have produced the patterns of inversion polymorphism we observe. Cline analysis revealed distinct changes in arrangement frequency between the ecotypes for all four inversions (Figures 5-6), indicating a role of divergent selection between habitats. Previous evidence for adaptive trait QTL and outlier SNPs on LG12 (Morales *et al.* 2019; Koch *et al.* 2021; Westram *et al.* 2021) supports this. LG12 contributes strongly to phenotypic variation in shape, aperture, and shell length (Koch *et al.* 2021), suggesting the presence of alleles under strong habitat-specific selection. Our analyses here also highlight a role for sex-specific selection in the Crab ecotype. At the moment, it is not clear whether the spread of the inversions was first promoted by divergent selection between ecotypes or by their role in sex chromosome evolution.

Several potential drivers for recombination suppression in the evolution of sex chromosomes have been discussed, including genetic drift, heterozygote advantage, and meiotic drive as well as sexual antagonism, but evidence distinguishing them remains scarce (Ironside 2010; Charlesworth 2017; Ponnikas *et al.* 2018). As the drift hypothesis requires small population sizes and heterozygote advantage is favoured in inbreeding populations, neither mechanism seems likely to explain the strong ecotype differences we observe. Sexually antagonistic selection remains the predominant theory for the evolution of sex chromosomes (Fisher 1931; Rice 1996) and it seems plausible for *L. saxatilis* because the effects of a trait on male and female fitness can depend on the local environment (Connallon and Clark 2014; Connallon 2015), potentially resulting in environment-dependent sexual antagonism. However, note that population differences in sex chromosomes can occur without invoking the need for varying sexual conflict (Bergero and Charlesworth 2019). If it is assumed that sexual antagonism did indeed play a role in the evolution of this young sex-determining region, at least two scenarios can be considered for the evolution of the LG12 putative inversions. In one, LGC12.2 first evolved in Crab females due to the presence of a locus with sexually antagonistic effects close to the sex-determining locus. The sexual dimorphism selected for in Crab was disadvantageous in Wave. The derived arrangement spread into Wave, for reasons unknown, and lost its association with sex through rare recombination events during interbreeding in the hybrid zone, which placed the Z allele at the sex-determining locus onto the derived background. Another scenario is possible where LGC12.2 first appeared in the Wave ecotype and spread because it enhanced local adaptation. Recombination allowed both Z and W alleles at the sex-determining locus to be present on the derived arrangement. Sexual antagonism was not the driver of the evolution of the putative inversion in this case; however, it remains necessary to explain the spread into Crab of only the derived arrangement carrying the female-specific (W) allele at the sex-determining locus. Both scenarios require disparate selection on males and females between the two ecotypes; some aspect of the Crab environment creates differential fitness effects of a trait for males and females, but this does not occur in the Wave habitat. One potential example of such a trait is size; size-dimorphism

between the sexes is more pronounced in Crab than Wave (Perini *et al.* 2020). In Crab, males mature early to enable mating as early as possible, whereas females need to create space for as many embryos as possible and therefore grow larger and mature later. However, in Wave a large size is selected against in both males and females as individuals must fit into small crevices for protection from waves.

These scenarios also assume that the same sex-determining locus is present in both Crab and Wave. Whether this is the case is unknown. If Wave does not share the LG12 sex-determining locus, many more different scenarios of selection are possible. For example, the putative inversion may have arisen and spread among the two ecotypes due to locally adaptive effects, as with other inversions in *L. saxatilis.* Subsequently, sexual antagonism in Crab led to a new female-determining allele arising within the derived arrangement in Crab, spreading to fixation on that arrangement and reducing its male-specific fitness such that the ancestral arrangement fixed in males. Again, the lack of sexual antagonism prevented the spread of this haplotype into Wave. This scenario has the advantage that it does not rely on unexplained spread of an arrangement between ecotypes and rare recombination to alter the relationship between the sex determining alleles and putative inversion arrangements. It predicts that sex is determined by a different locus in Wave and our analyses suggest that this locus could be on LG5.

A similar pattern of selection is required to explain the evolution of LGC12.3. The strong association with sex and predominant presence of the derived arrangement in Crab females only, supports a role of sexually antagonistic selection in Crab to create a second stratum of a sex-determining region. Again, a strong barrier to spread of the derived arrangement into Wave must exist since most Wave individuals are homozygous for the ancestral arrangement. Capture of locally adaptive loci may be involved in the maintenance of this barrier; however, the strong sex-association means it is improbable that divergent natural selection alone would produce the observed ecotype differences. The clinal variation in arrangements of LGC12.4 but weak sex-association gives weight to the possibility that this inversion is predominantly involved in ecotypic rather than sex differentiation.

The disparity between the ecotypes in the emergence of a sex-determining region is striking. Populations are only a few metres apart and readily interbreed in the hybrid zone. There is no evidence for substantial periods of allopatric divergence (Butlin *et al.* 2014). The distinct barrier to the spread of the sex-determining region from the Crab ecotype into Wave indicates that there must be a difference in the selective regime acting on the two ecotypes. While clearly very complex and not yet fully understood, this undoubtedly must involve sex-specific selection as well as the divergent natural selection previously characterised in *L. saxatilis.* Further analysis of this system, including of additional hybrid zones across Europe, will aid understanding of this intricate pattern and is likely to give new insights into both local adaptation and sex chromosome evolution.

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2.6 Data Accessibility

No new data were generated for these analyses. See Westram *et al.* (2018) and Koch *et al.* (2021) for access to previously published data. Analysis scripts, and data files saved at intermediate steps for simplicity, are available on GitHub at https://github.com/katiehearn/Littorina_sex_ANG.

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2.8 Supplementary Methods

Inversion detection using LDNA and PCA

The two main parameters in LDNA, |E|min and φ, were manipulated to investigate the detection of clusters. |E|min represents the minimum number of edges required for a cluster to be an outlier, where edges are connections between pairs of SNPs that are in LD above a threshold. This is correlated to some extent with the number of SNPs in a cluster. The other parameter, φ, relates to the minimum LD threshold for a cluster to be considered an outlier; it compares the median intracluster pairwise LD to intercluster LD. A greater value of φ indicates that the cluster 'stands out' more against the background LD. Various combinations of the two parameters were tested, with |E|min between 30 and 4000 and φ between 1 and 10, and the output tables of putative outlier clusters saved for further investigation.

Low values of the two parameters are less 'stringent' so many more clusters were retained in the output. Clusters with few edges are more likely to be small clusters of SNPs in close physical linkage. Clusters with a median pairwise LD below 0.3 were excluded since loci in inversions are expected to be under high LD. The distributions of SNPs in clusters were checked, as SNPs in clusters representing inversions are expected to be located in one region of the linkage group rather than scattered. The identities of SNPs in clusters were also checked as one large cluster detected under a certain combination of parameters was often detected as a few smaller clusters under different parameter combinations.

The set of clusters that was repeatedly detected under numerous different parameter combinations, and passed the above checks, was retained for downstream principal component analysis. The first two principal components were examined for the distinctive three groups on PC1 that indicate individuals that are homokaryotypic for each inversion arrangement, or heterokaryotypic. Distinct clusters on PC1 without intermediates are produced due to the lack of recombination between alternate arrangements, which allows allele frequencies to diverge between arrangements for many SNPs. The central cluster represents heterokaryotypes since they hold intermediate allele frequencies. Individuals were manually assigned a genotype (hom1, het, hom2) based on their PC1 grouping; the small numbers of individuals that were not clearly part of a cluster were assigned an N/A value.

Testing the effect of genotype, sex, and ecotype on π

Any group with fewer than two individuals was excluded (see Supplementary Table 4 for the number of snails in each group). Groups were also reordered to ensure the group taken as the intercept by the model was not empty; the Wave female heterozygote group (F_RA_W) was used as the intercept for all inversions. Values were log-transformed to give normally distributed data before models were fitted. Map position was included as a random effect as contigs at the same map position are not independent. Random slopes and intercepts were included in the model. The full model (which includes all fixed effects and interactions) was used as the global model in the MuMIn dredge() function to determine the best fitting model. This function tested all combinations of fixed effects and interactions. Only models with a ΔAIC<2 compared to the best fitting model were retained. For inversions where only one model was retained, estimates were extracted. Where more than one model was retained for a putative inversion, models were averaged using the MuMIn function model.avg() before the model-weighted average estimates were extracted.

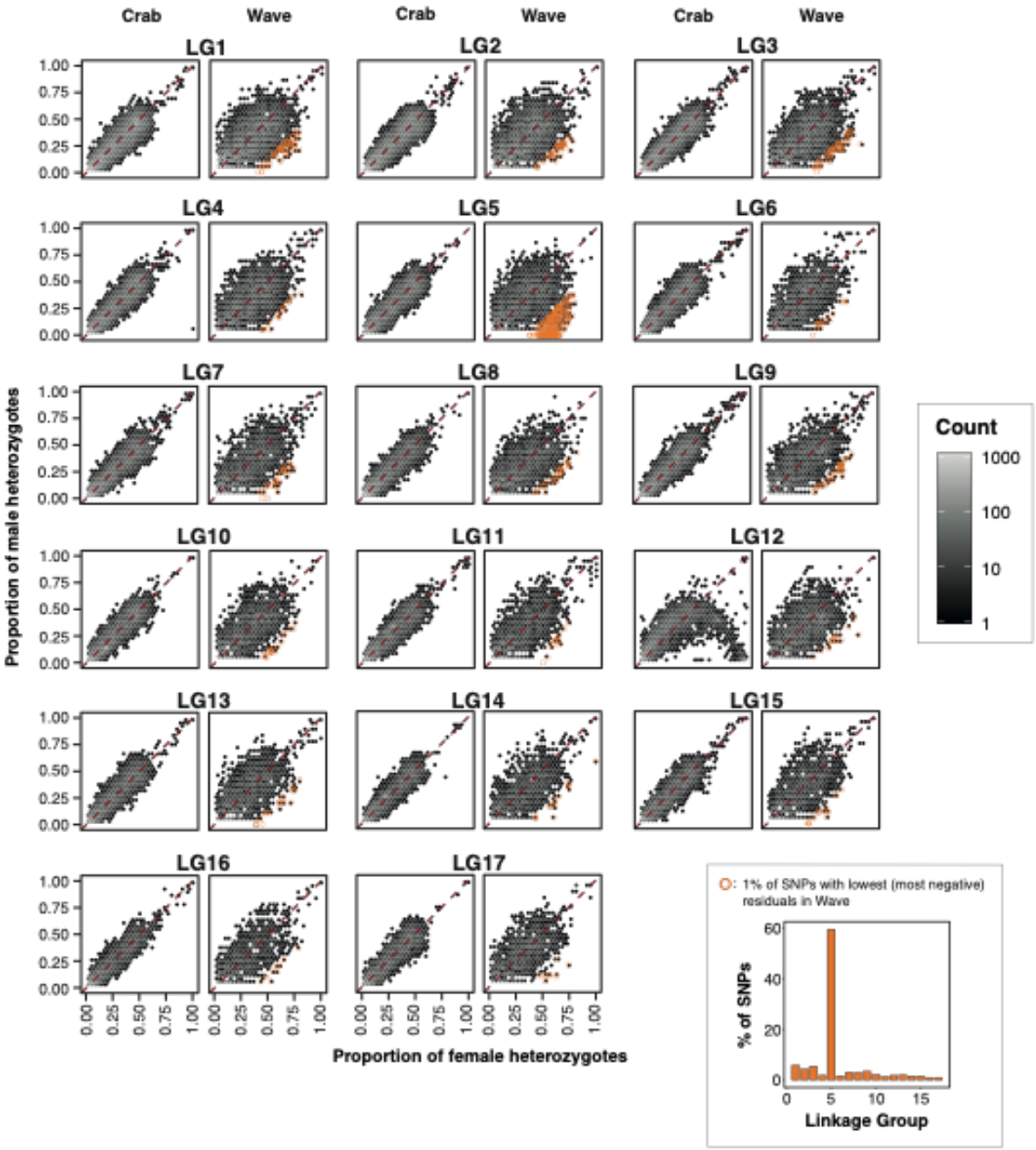
2.9 Supplementary Results

Inversion detection

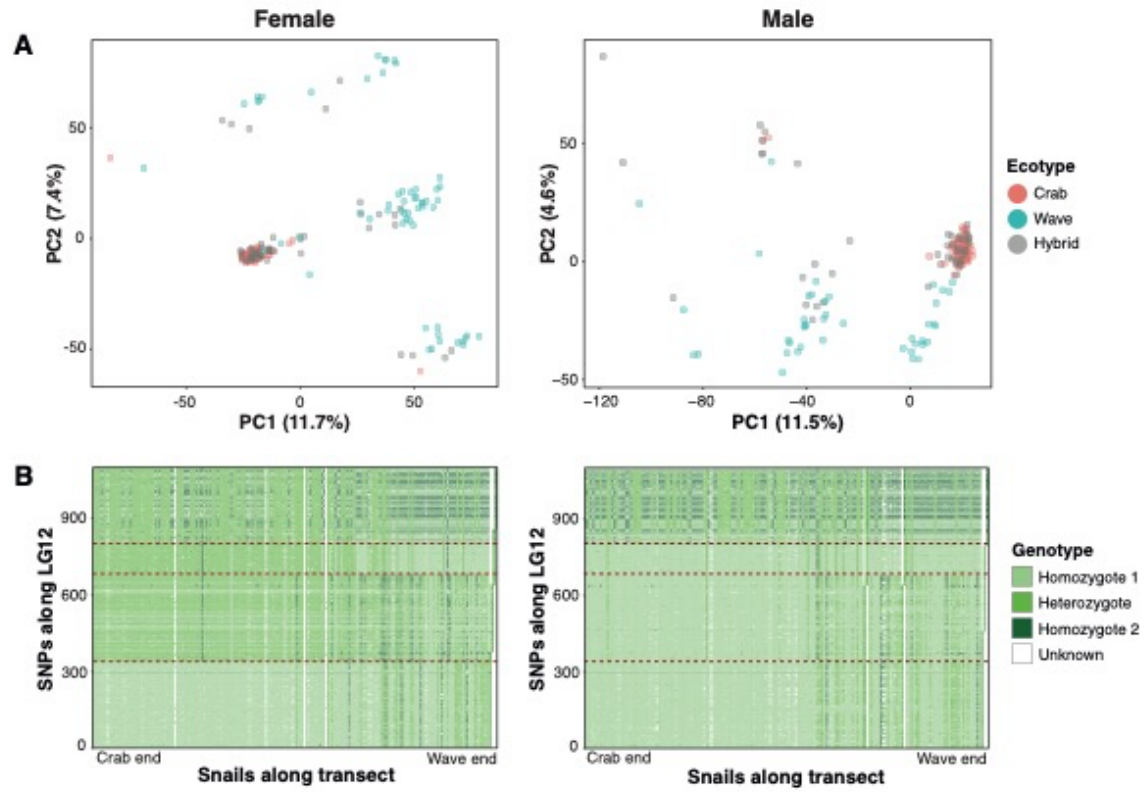
Many 'outlier clusters' were detected in the LDNA investigation (Supplementary Table 1). Only five clusters appeared repeatedly at all parameter combinations, while others only appeared at certain parameter values or did not meet minimum threshold requirements or other checks. This was especially notable at high values of |E|min and φ (end of Supplementary Table 1), where the outputs consisted mostly of the five clusters. Small clusters appearing at lower parameter values were mostly just small subsets of the five clusters. As a result, these five clusters were selected for continued analysis.

The distribution of SNPs in the clusters along LG12 was plotted (Figure 3b). One cluster covered the first half of the linkage group and another the end section, but the other three clusters had overlapping distributions in the central region of LG12. The three clusters all started from the same point on LG12, but one cluster covered a longer region of the linkage group than the other two clusters. A PCA of the central region covered by the three clusters revealed 6 groups separated by PC1 and PC2 (Supplementary Figure 2a). This is indicative of two inversions in LD with each other, as each cluster represents one of the six combinations possible of the three genotypes at the two inversions, overlapping or in LD. This was supported by examining haplotypes of individuals along LG12 (Supplementary Figure 2b); the central region covered by the three LDNA clusters was clearly split into two regions with differing haplotypes. The map position where the central region haplotypes split was the same as the point where two of the three central LDNA clusters end. It was therefore clear that the three LDNA clusters in the centre of LG12 represented two adjacent inversions. Non-random association of genotypes for the two inversions probably explains the generation of overlapping LDNA clusters. From this point on, these two regions were used for analysis in addition to the two regions represented by the LDNA clusters at the start and end of LG12.

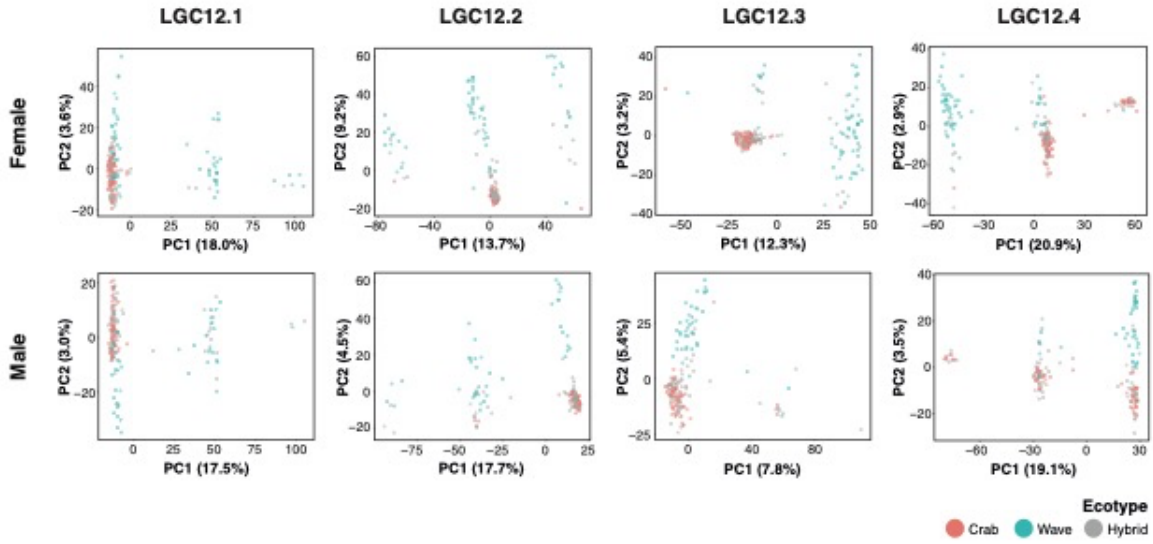
2.10 Supplementary Figures



Supplementary Figure 1. The proportions of each sex that are heterozygous at SNPs on each of the 17 linkage groups in the two ecotypes. Hexagons with a greater density of SNPs are shaded lighter grey. SNPs with a greater difference in heterozygosity between the sexes are further from the 1:1 line (neutral expectation of equal heterozygote proportions between the sexes). The 1% of SNPs with the most negative residuals in Wave are marked by orange circles; the insert shows the distribution of these SNPs across the 17 linkage groups.

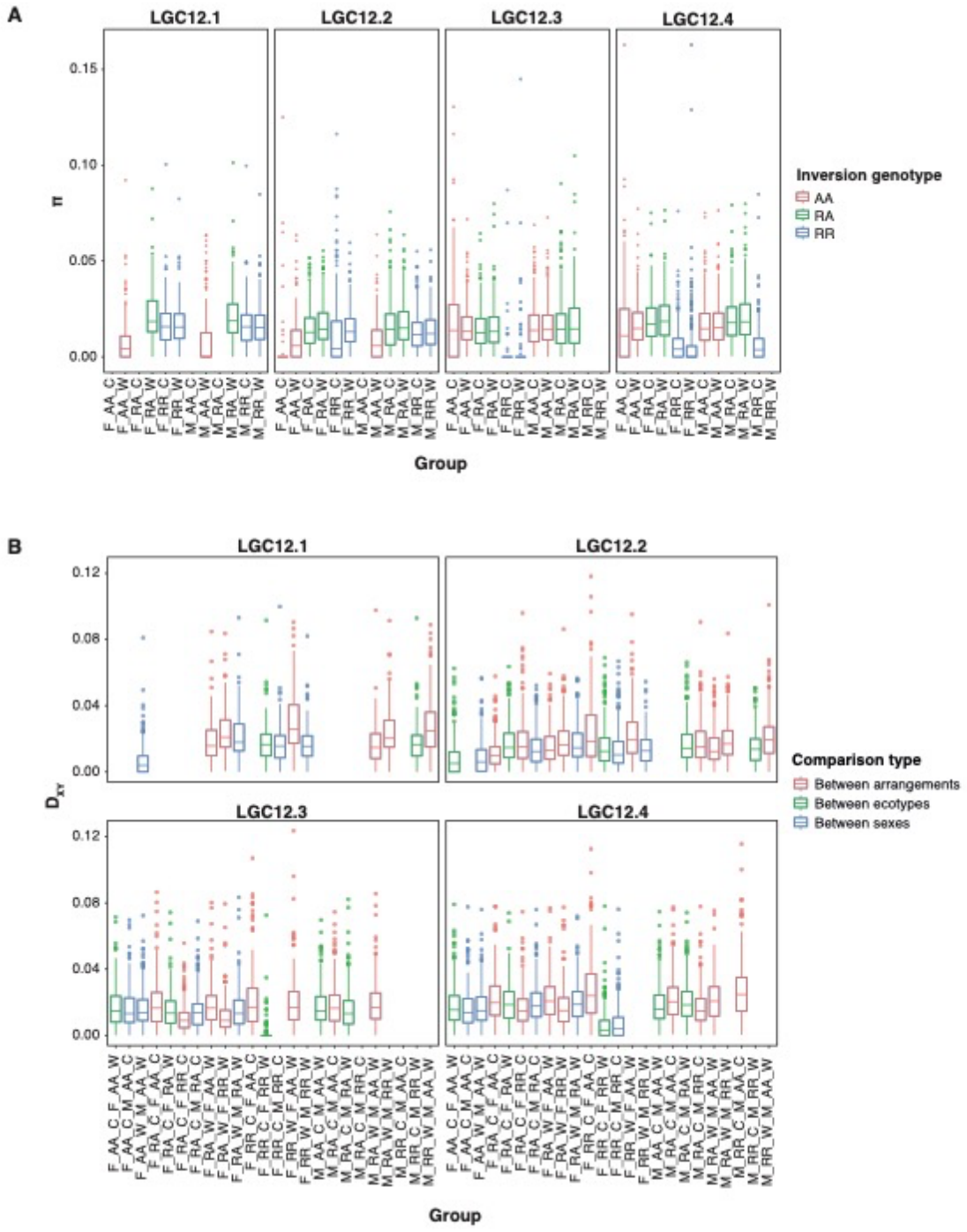


Supplementary Figure 2. A) PC1 vs PC2 of a PCA of SNPs in the central region of LG12 covered by three LD clusters, for females and males. **B)** Plot showing the SNP genotypes along LG12 for every individual (each column shows an individual's haplotype), in order along the sampling transect, for females and males. Red horizontal dashed lines denote the four different regions of LG12 (Figure 2)- the central region of LG12 used in **A)** corresponds to the two central sections in **B)**.



Supplementary Figure 3. PC1 vs PC2 of a PCA of SNPs for all individuals (both sexes and ecotypes) for each putative inversion- LGC12.1 (0-32.8cM); LGC12.2 (33.0-43.8cM);

LGC12.3 (43.8-48.7cM); and LGC12.4 (48.7-60.0cM). The three distinct clusters along PC1 that represent the three inversion genotypes are visible for all regions.



Supplementary Figure 4. π **(A)** and d_{XY} **(B)** per contig in/between groups of individuals of each combination of inversion genotype, ecotype and sex for the four inversions. The first letter of each x axis label refers to the sex (F- female; M- male); the second part refers to the genotype (AA homozygote; RA heterozygote; RR homozygote); and the last letter refers to the ecotype (C- Crab; W- Wave) for each group. In **(B)**, labels include the two group codes for the two groups in each d_{XY} comparison.

2.11 Supplementary Tables

Supplementary Table 1. Full LDna cluster outputs for varying combinations of the input parameters phi (φ) and minimum number of edges (|E|min) (columns one and two). Outputs are ordered in ascending values of |E|min and phi. The five clusters of interest are shaded in grey every time they appear.

Edges	Phi	Cluster name	Merge.at	nLoci	nE	lambda	Median.LD	MAD.LD
30	1	1509_0.96	0.95	133	5085	3.99	0.964	0.024
30	1	1837_0.94	0.93	207	10310	4.14	0.932	0.0416
30	1	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
30	1	2046_0.92	0.91	183	4899	3.66	0.868	0.0648
30	1	2329_0.89	0.86	10	38	4.9	0.977	0.0121
30	1	2406_0.88	0.86	31	194	8.37	0.85	0.14
30	1	2640_0.85	0.6	23	232	20.47	0.97	0.0148
30	1	2849_0.81	0.6	12	34	7.56	0.807	0.074
30	1	2927_0.79	0.74	19	160	3.8	0.978	0.0169
30	1	2943_0.79	0.52	18	107	12.78	0.921	0.06
30	1	3026_0.77	0.73	36	189	4.32	0.673	0.127
30	1	3217_0.73	0.71	30	165	4.5	0.659	0.116
30	1	3228_0.73	0.66	16	58	7.44	0.696	0.178
30	1	3243_0.73	0.54	13	39	9.035	0.702	0.105
30	1	3334_0.71	0.64	30	146	6.9	0.611	0.151
30	1	3517_0.67	0.59	16	62	3.92	0.704	0.162
30	1	3596_0.65	0.62	22	106	11.44	0.62	0.15
30	1	3608_0.65	0.48	11	32	8.36	0.768	0.182
30	1	3653_0.64	0.49	10	37	9.7	0.98	0.0129
30	1	3671_0.63	0.62	36	124	10.08	0.423	0.141
30	1	3674_0.63	0.62	11	32	4.235	0.745	0.17
30	1	3693_0.63	0.51	13	39	7.605	0.618	0.162
30	1	3736_0.62	0.48	10	37	6.7	0.936	0.0508
30	1	3767_0.61	0.59	17	53	6.46	0.527	0.226
30	1	3852_0.59	0.58	26	96	10.92	0.445	0.179
30	1	3888_0.59	0.37	18	81	11.88	0.674	0.112
30	1	3942_0.57	0.56	24	91	11.4	0.487	0.189
30	1	3944_0.57	0.56	19	55	6.46	0.38	0.17
30	1	4010_0.56	0.51	16	48	5.12	0.506	0.126
30	1	4050_0.55	0.49	17	36	5.27	0.362	0.0957
30	1	4058_0.55	0.46	12	35	4.26	0.555	0.322
30	1	4074_0.55	0.29	11	44	7.59	0.702	0.153
30	1	4105_0.54	0.49	15	71	4.65	0.72	0.204
30	1	4127_0.54	0.29	15	63	9	0.614	0.168

30	1	4146_0.53	0.51	31	151	4.34	0.409	0.121
30	1	4195_0.52	0.49	15	35	4.8	0.348	0.175
30	1	4241_0.51	0.47	14	57	9.1	0.657	0.203
30	1	4253_0.51	0.43	18	37	3.96	0.233	0.149
30	1	4257_0.51	0.42	17	97	11.05	0.649	0.299
30	1	4266_0.51	0.33	10	30	7.3	0.732	0.245
30	1	4274_0.5	0.49	11	30	3.74	0.527	0.21
30	1	4278_0.5	0.48	45	214	4.5	0.332	0.119
30	1	4384_0.48	0.44	10	37	4.9	0.642	0.156
30	1	4395_0.48	0.42	15	44	6.75	0.451	0.376
30	1	4432_0.47	0.43	22	59	5.28	0.248	0.238
30	1	4467_0.46	0.45	16	36	5.44	0.356	0.154
30	1	4563_0.44	0.42	12	38	5.22	0.467	0.103
30	1	4618_0.43	0.38	16	75	10.08	0.638	0.227
30	1	4653_0.42	0.37	18	36	4.14	0.228	0.144
30	1	4663_0.42	0.32	16	53	5.92	0.369	0.142
30	1	4679_0.41	0.39	12	55	7.8	0.656	0.247
30	1	4708_0.41	0.24	18	62	6.84	0.378	0.301
30	1	4714_0.4	0.39	11	32	5.115	0.506	0.475
30	1	4730_0.4	0.34	16	44	5.92	0.369	0.322
30	1	4750_0.39	0.38	18	43	3.6	0.209	0.179
30	1	4757_0.39	0.37	32	82	5.12	0.168	0.0951
30	1	4860_0.36	0.35	27	95	6.21	0.238	0.128
30	1	4872_0.36	0.31	11	42	3.85	0.363	0.147
30	1	4998_0.31	0.29	12	38	4.62	0.397	0.396
30	2	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
30	2	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
30	2	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
30	2	2406_0.88	0.86	31	194	8.37	0.85	0.14
30	2	2640_0.85	0.6	23	232	20.47	0.97	0.0148
30	2	2667_0.84	0.83	246	10952	9.84	0.772	0.117
30	2	2849_0.81	0.6	12	34	7.56	0.807	0.074
30	2	2943_0.79	0.52	18	107	12.78	0.921	0.06
30	2	3228_0.73	0.66	16	58	7.44	0.696	0.178
30	2	3243_0.73	0.54	13	39	9.035	0.702	0.105
30	2	3334_0.71	0.64	30	146	6.9	0.611	0.151
30	2	3415_0.69	0.68	57	273	10.26	0.49	0.117
30	2	3469_0.68	0.64	32	202	8.32	0.623	0.266
30	2	3508_0.67	0.65	62	402	17.36	0.442	0.161
30	2	3596_0.65	0.62	22	106	11.44	0.62	0.15
30	2	3608_0.65	0.48	11	32	8.36	0.768	0.182
30	2	3653_0.64	0.49	10	37	9.7	0.98	0.0129
30	2	3671_0.63	0.62	36	124	10.08	0.423	0.141

30	2	3693_0.63	0.51	13	39	7.605	0.618	0.162
30	2	3736_0.62	0.48	10	37	6.7	0.936	0.0508
30	2	3767_0.61	0.59	17	53	6.46	0.527	0.226
30	2	3852_0.59	0.58	26	96	10.92	0.445	0.179
30	2	3888_0.59	0.37	18	81	11.88	0.674	0.112
30	2	3942_0.57	0.56	24	91	11.4	0.487	0.189
30	2	3944_0.57	0.56	19	55	6.46	0.38	0.17
30	2	3995_0.56	0.54	25	131	6.25	0.473	0.219
30	2	4050_0.55	0.49	17	36	5.27	0.362	0.0957
30	2	4074_0.55	0.29	11	44	7.59	0.702	0.153
30	2	4127_0.54	0.29	15	63	9	0.614	0.168
30	2	4241_0.51	0.47	14	57	9.1	0.657	0.203
30	2	4257_0.51	0.42	17	97	11.05	0.649	0.299
30	2	4266_0.51	0.33	10	30	7.3	0.732	0.245
30	2	4280_0.5	0.48	30	88	8.1	0.306	0.144
30	2	4340_0.49	0.42	21	95	8.61	0.43	0.415
30	2	4395_0.48	0.42	15	44	6.75	0.451	0.376
30	2	4432_0.47	0.43	22	59	5.28	0.248	0.238
30	2	4463_0.46	0.45	71	334	13.49	0.192	0.124
30	2	4467_0.46	0.45	16	36	5.44	0.356	0.154
30	2	4470_0.46	0.44	61	333	14.64	0.247	0.108
30	2	4520_0.45	0.43	61	186	6.1	0.114	0.113
30	2	4618_0.43	0.38	16	75	10.08	0.638	0.227
30	2	4663_0.42	0.32	16	53	5.92	0.369	0.142
30	2	4679_0.41	0.39	12	55	7.8	0.656	0.247
30	2	4708_0.41	0.24	18	62	6.84	0.378	0.301
30	2	4730_0.4	0.34	16	44	5.92	0.369	0.322
30	2	4860_0.36	0.35	27	95	6.21	0.238	0.128
30	5	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
30	5	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
30	5	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
30	5	2640_0.85	0.6	23	232	20.47	0.97	0.0148
30	5	2943_0.79	0.52	18	107	12.78	0.921	0.06
30	5	3414_0.69	0.68	347	21468	34.7	0.579	0.177
30	5	3508_0.67	0.65	62	402	17.36	0.442	0.161
30	5	3596_0.65	0.62	22	106	11.44	0.62	0.15
30	5	3852_0.59	0.58	26	96	10.92	0.445	0.179
30	5	3888_0.59	0.37	18	81	11.88	0.674	0.112
30	5	3942_0.57	0.56	24	91	11.4	0.487	0.189
30	5	4257_0.51	0.42	17	97	11.05	0.649	0.299
30	5	4463_0.46	0.45	71	334	13.49	0.192	0.124
30	5	4470_0.46	0.44	61	333	14.64	0.247	0.108
30	5	4583_0.44	0.35	72	973	23.76	0.337	0.173

30	10	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
30	10	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
30	10	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
30	10	2640_0.85	0.6	23	232	20.47	0.97	0.0148
30	10	3414_0.69	0.68	347	21468	34.7	0.579	0.177
30	10	4583_0.44	0.35	72	973	23.76	0.337	0.173
60	5	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
60	5	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
60	5	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
60	5	2640_0.85	0.6	23	232	20.47	0.97	0.0148
60	5	2943_0.79	0.52	18	107	12.78	0.921	0.06
60	5	3414_0.69	0.68	347	21468	34.7	0.579	0.177
60	5	3508_0.67	0.65	62	402	17.36	0.442	0.161
60	5	3596_0.65	0.62	22	106	11.44	0.62	0.15
60	5	3888_0.59	0.37	18	81	11.88	0.674	0.112
60	5	3942_0.57	0.56	24	91	11.4	0.487	0.189
60	5	4257_0.51	0.42	17	97	11.05	0.649	0.299
60	5	4463_0.46	0.45	71	334	13.49	0.192	0.124
60	5	4470_0.46	0.44	61	333	14.64	0.247	0.108
60	5	4583_0.44	0.35	72	973	23.76	0.337	0.173
100	10	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
100	10	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
100	10	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
100	10	3414_0.69	0.68	347	21468	34.7	0.579	0.177
100	10	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	1	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
200	1	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	1	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	1	2401_0.88	0.87	221	7954	4.42	0.816	0.095
200	1	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	1	3415_0.69	0.68	57	273	10.26	0.49	0.117
200	1	3469_0.68	0.64	32	202	8.32	0.623	0.266
200	1	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	1	4239_0.51	0.49	40	218	5.8	0.235	0.227
200	1	4240_0.51	0.48	59	273	8.26	0.165	0.165
200	1	4278_0.5	0.48	45	214	4.5	0.332	0.119
200	1	4470_0.46	0.44	61	333	14.64	0.247	0.108
200	2	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
200	2	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	2	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	2	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	2	2667_0.84	0.83	246	10952	9.84	0.772	0.117
200	2	3415_0.69	0.68	57	273	10.26	0.49	0.117

200	2	3469_0.68	0.64	32	202	8.32	0.623	0.266
200	2	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	2	4240_0.51	0.48	59	273	8.26	0.165	0.165
200	2	4463_0.46	0.45	71	334	13.49	0.192	0.124
200	2	4470_0.46	0.44	61	333	14.64	0.247	0.108
200	3	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
200	3	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	3	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	3	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	3	2667_0.84	0.83	246	10952	9.84	0.772	0.117
200	3	3415_0.69	0.68	57	273	10.26	0.49	0.117
200	3	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	3	4463_0.46	0.45	71	334	13.49	0.192	0.124
200	3	4470_0.46	0.44	61	333	14.64	0.247	0.108
200	3	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	4	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
200	4	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	4	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	4	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	4	3414_0.69	0.68	347	21468	34.7	0.579	0.177
200	4	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	4	4463_0.46	0.45	71	334	13.49	0.192	0.124
200	4	4470_0.46	0.44	61	333	14.64	0.247	0.108
200	4	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	5	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
200	5	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	5	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	5	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	5	3414_0.69	0.68	347	21468	34.7	0.579	0.177
200	5	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	5	4463_0.46	0.45	71	334	13.49	0.192	0.124
200	5	4470_0.46	0.44	61	333	14.64	0.247	0.108
200	5	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	6	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
200	6	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	6	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	6	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	6	3414_0.69	0.68	347	21468	34.7	0.579	0.177
200	6	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	6	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	7	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
200	7	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	7	2398_0.88	0.87	268	17510	91.12	0.871	0.0748

200	7	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	7	3414_0.69	0.68	347	21468	34.7	0.579	0.177
200	7	3508_0.67	0.65	62	402	17.36	0.442	0.161
200	7	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	8	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
200	8	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	8	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	8	2640_0.85	0.6	23	232	20.47	0.97	0.0148
200	8	3414_0.69	0.68	347	21468	34.7	0.579	0.177
200	8	4583_0.44	0.35	72	973	23.76	0.337	0.173
200	10	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
200	10	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
200	10	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
200	10	3414_0.69	0.68	347	21468	34.7	0.579	0.177
400	1	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
400	1	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	1	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	1	2667_0.84	0.83	246	10952	9.84	0.772	0.117
400	1	3508_0.67	0.65	62	402	17.36	0.442	0.161
400	1	3658_0.64	0.44	62	429	6.2	0.43	0.162
400	2	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
400	2	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	2	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	2	2667_0.84	0.83	246	10952	9.84	0.772	0.117
400	2	3508_0.67	0.65	62	402	17.36	0.442	0.161
400	2	4583_0.44	0.35	72	973	23.76	0.337	0.173
400	3	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
400	3	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	3	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	3	2667_0.84	0.83	246	10952	9.84	0.772	0.117
400	3	3508_0.67	0.65	62	402	17.36	0.442	0.161
400	3	4583_0.44	0.35	72	973	23.76	0.337	0.173
400	4	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
400	4	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	4	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	4	3414_0.69	0.68	347	21468	34.7	0.579	0.177
400	4	3508_0.67	0.65	62	402	17.36	0.442	0.161
400	4	4583_0.44	0.35	72	973	23.76	0.337	0.173
400	5	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
400	5	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	5	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	5	3414_0.69	0.68	347	21468	34.7	0.579	0.177
400	5	3508_0.67	0.65	62	402	17.36	0.442	0.161

400	5	4583_0.44	0.35	72	973	23.76	0.337	0.173
400	6	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
400	6	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	6	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	6	3414_0.69	0.68	347	21468	34.7	0.579	0.177
400	6	3508_0.67	0.65	62	402	17.36	0.442	0.161
400	6	4583_0.44	0.35	72	973	23.76	0.337	0.173
400	7	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
400	7	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	7	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	7	3414_0.69	0.68	347	21468	34.7	0.579	0.177
400	7	4583_0.44	0.35	72	973	23.76	0.337	0.173
400	8	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
400	8	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
400	8	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
400	8	3414_0.69	0.68	347	21468	34.7	0.579	0.177
400	8	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	1	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
600	1	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	1	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	1	2667_0.84	0.83	246	10952	9.84	0.772	0.117
600	1	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	2	2043_0.92	0.91	124	3950	8.68	0.921	0.0534
600	2	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	2	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	2	2667_0.84	0.83	246	10952	9.84	0.772	0.117
600	2	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	3	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
600	3	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	3	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	3	2667_0.84	0.83	246	10952	9.84	0.772	0.117
600	3	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	4	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
600	4	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	4	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	4	3414_0.69	0.68	347	21468	34.7	0.579	0.177
600	4	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	5	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
600	5	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	5	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	5	3414_0.69	0.68	347	21468	34.7	0.579	0.177
600	5	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	6	2317_0.89	0.88	199	5105	39.8	0.811	0.0719

600	6	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	6	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	6	3414_0.69	0.68	347	21468	34.7	0.579	0.177
600	6	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	7	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
600	7	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	7	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	7	3414_0.69	0.68	347	21468	34.7	0.579	0.177
600	7	4583_0.44	0.35	72	973	23.76	0.337	0.173
600	8	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
600	8	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
600	8	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
600	8	3414_0.69	0.68	347	21468	34.7	0.579	0.177
600	8	4583_0.44	0.35	72	973	23.76	0.337	0.173
1000	10	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
1000	10	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
1000	10	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
1000	10	3414_0.69	0.68	347	21468	34.7	0.579	0.177
4000	4	2317_0.89	0.88	199	5105	39.8	0.811	0.0719
4000	4	2318_0.89	0.88	222	10937	55.5	0.87	0.0705
4000	4	2398_0.88	0.87	268	17510	91.12	0.871	0.0748
4000	4	3414_0.69	0.68	347	21468	34.7	0.579	0.177

Supplementary Table 2. Cline parameter estimates and confidence intervals (C.I.s) for the best cline model of R arrangement frequency along the transect for each of the four inversions for males and females. Crab frequency and Wave frequency indicate the fitted frequencies of the R arrangement in the Crab and the Wave ends of the transect. Best cline model was taken as the model with the lowest AIC value (see Supplementary Table 3).

Inversion	Sex	Best model	Crab frequency			Wave frequency			Centre (m)			Width (m)		
			Estimate	Lower C.I.	Upper C.I.	Estimate	Lower C.I.	Upper C.I.	Estimate	Lower C.I.	Upper C.I.	Estimate	Lower C.I.	Upper C.I.
12.1	Female	Full	0.996	0.981	-	0.720	0.630	0.798	87.589	82.853	95.606	6.08	0.729	34.865
	Male	Full	1.000	1.00	-	0.750	0.674	0.817	85.118	82.031	85.666	0.104	0.00	-
12.2	Female	No cline	0.510	0.461	0.558	0.510	0.461	0.558	N/A	N/A	N/A	N/A	N/A	N/A
	Male	Full	0.983	-	-	0.663	-	-	83.761	-	-	4.286	-	-
12.3	Female	Wave-constrained	0.503	0.434	0.597	0.042	0.024	0.066	90.329	83.648	96.270	23.000	0.476	54.298
	Male	Wave-constrained	0.042	0.024	0.066	0.042	0.024	0.066	90.329	83.648	96.270	23.000	0.476	54.298
12.4	Female	Wave-constrained	0.603	0.517	0.666	0.081	0.043	0.132	90.433	87.613	93.917	10.122	0.758	21.155
	Male	Wave-constrained	0.326	0.268	0.389	0.081	0.043	0.132	90.433	87.613	93.917	10.122	0.758	21.155

Supplementary Table 3. AIC values for the full cline models and the four alternative models for each inversion. For models fitted separately to males and females ('full model' and 'no cline'), the sum of male and female AIC values for each combination of these is included. The model (or combination of models) with the lowest AIC value for males and females for each inversion is highlighted in bold.

Inversion	Male + female 'full model'	Male + female 'no cline'	Male 'full model' + female 'no cline'	Female 'full model' + male 'no cline'	'Combined' cline	'Constrained' cline	'Wave-constrained' cline
12.1	**284.61**	406.58	347.49	345.70	285.04	288.08	N/A
12.2	516.26	570.70	**510.57**	576.42	667.80	512.37	516.72
12.3	381.30	440.40	438.65	383.05	522.35	384.50	**381.05**
12.4	599.04	694.59	674.06	619.57	634.06	597.57	**596.31**

Supplementary Table 4. The number of individuals of each sex and ecotype of each inversion genotype for the four inversions. '-' indicates zero snails.

Inversion	Sex	RR		RA		AA	
		Crab	Wave	Crab	Wave	Crab	Wave
12.1	Female	90	31	-	22	-	5
	Male	62	23	-	18	-	1
12.2	Female	1	12	88	32	1	14
	Male	60	17	2	21	-	4
12.3	Female	1	1	88	10	1	47
	Male	-	-	2	3	60	39
12.4	Female	22	1	66	13	1	44
	Male	7	-	26	6	29	36

Supplementary Table 5. The best fitting models of the effect of various factors on π (any with ΔAIC<2 compared to the best model) for each of the four inversions, and weighting when models are averaged.

Inversion	Ecotype	Genotype	Sex	Ecotype: genotype	Ecotype: sex	Genotype: sex	Ecotype: genotype:sex	df	AICc	ΔAIC	Weight
LGC12.1		+						82	2650.240	0	1
LGC12.2	+	+	+	+	+			86	4975.964	0	1
LGC12.3		+	+			+		41	3848.390	0	0.419
LGC12.3	+	+	+		+	+		43	3849.118	0.728	0.291
LGC12.3	+	+	+			+		42	3849.123	0.734	0.290
LGC12.4		+						82	5467.944	0	0.320
LGC12.4	+	+						83	5468.456	0.511	0.247
LGC12.4	+	+	+					84	5469.345	1.401	0.159
LGC12.4		+	+					83	5469.516	1.571	0.146
LGC12.4	+	+		+				84	5469.764	1.819	0.129

Supplementary Table 6. Model estimates for the best fitting model of the effect of various factors on π for each inversion. Model-weighted average estimates are presented for inversions with more than one best fitting model (see Supplementary Table 5). Estimates and standard errors are for log-transformed data.

Inversion	Coefficients	Estimate	Standard Error
LGC12.1	Intercept	-3.9759	0.0702
LGC12.1	GenotypeAA	-0.8303	0.1197
LGC12.1	GenotypeRR	-0.2728	0.0823
LGC12.2	Intercept	-4.2778	0.0656
LGC12.2	EcotypeC	-0.2615	0.0681
LGC12.2	GenotypeAA	-0.4179	0.1024
LGC12.2	GenotypeRR	-0.2011	0.0543
LGC12.2	SexM	-0.0021	0.0450
LGC12.2	EcotypeC:genotypeRR	-0.2933	0.0977
LGC12.2	EcotypeC:sexM	0.4088	0.0940
LGC12.3	Intercept	-4.4040	0.0718
LGC12.3	GenotypeAA	0.0580	0.0805
LGC12.3	SexM	0.2584	0.0776

LGC12.3	GenotypeAA:sexM	-0.2356	0.0957
LGC12.3	EcotypeC	-0.0742	0.0911
LGC12.3	EcotypeC:sexM	0.0429	0.0852
LGC12.4	Intercept	-4.0724	0.0685
LGC12.4	GenotypeAA	-0.2680	0.0586
LGC12.4	GenotypeRR	-0.9312	0.1368
LGC12.4	EcotypeC	-0.0380	0.0477
LGC12.4	SexM	0.0120	0.0272
LGC12.4	EcotypeC:genotypeAA	0.0111	0.0403

CHAPTER THREE
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Population- and habitat- specific variation in sex determination and sex-linked inversions in *Littorina saxatilis*

3.1 Abstract

Sex determination systems and sex chromosomes are labile and highly diverse within and between species, but the underlying drivers and mechanisms are poorly understood. Divergent natural selection between populations can drive differentiation and reproductive isolation. Likewise, sex-specific selection may play an important role when it differs over heterogenous environments and with ecology, through its joint impact on sexual dimorphism and sex-specific local adaptation. The same selection pressures might drive sex and population differentiation simultaneously, highlighting the non-independence of the processes and the importance of studies that examine both concurrently. In particular, species with labile sex determination across populations will be valuable for comparative studies.

Local adaptation with gene flow and its genomic basis are well-studied in the intertidal snail, *Littorina saxatilis.* A sex-determination system has recently been discovered in one population that appears variable and differently associated with multiple inversions on linkage group 12 (LG12) across a hybrid zone between ecotypes. Therefore, in this study, six additional hybrid zones in three populations were studied to test for presence of the sex determination system and sex-linked inversions, and any variation between populations.

I show evidence for the same sex determination system across all populations. Three of the four inversions are present throughout, with the fourth restricted to one of three populations. Associations between sex and SNP genotypes and inversions are highly variable across populations and ecotypes, although the LGC12.2 inversion is always important in sex in the Crab ecotype. Inconsistent association of genotypes with sex are detected on LG12 and additional linkage groups in Wave, predominantly LG5. In conjunction, these patterns support a multigenic sex determination system with loci that contribute differently across ecotypes and populations. Incomplete associations of inversions with sex across environments highlight their role in both sex determination and adaptive divergence, and provide a valuable system with which to untangle their separate and joint effects in future genomic studies.

3.2 Introduction

There is a great diversity of sex chromosomes and sex determination systems across nature (Bachtrog *et al*. 2014; Furman *et al*. 2020). This variation extends both between and within species, and similar systems have evolved independently multiple times (Wright *et al*. 2016). Sexual antagonism, wherein sexes have differing optima for a trait and so experience opposing fitness effects, remains the most common theory for explaining why sex chromosomes evolve (Fisher 1931; Rice 1987; Rice 1996; Wright *et al*. 2016), although empirical evidence in support of it remains scarce (Ponnikas *et al*. 2018). Indeed, many questions remain unanswered about switches in sex-determination systems and around the

early mechanisms and drivers of sex chromosome evolution due to difficulties in studying them (Pennell *et al*. 2018; Vicoso 2019; Ramos and Antunes 2022).

In particular, the vast heterogeneity of sex determination systems between and within species is surprising given their importance in the fundamental traits of phenotypic sex (Bachtrog *et al*. 2014). Although many general patterns in sex determination and sex chromosome evolution have been identified, equal numbers of exceptions to these have emerged (Furman *et al*. 2020; Lenormand and Roze 2022). The lack of 'one rule' for these processes is a large contributor to the multitude of outstanding questions, as patterns are not conserved and so cannot be extrapolated across species or even populations. As a result, many more studies on sex determination across a variety of groups are needed.

In many cases, it is not understood why differences in sex determination systems exist between species or populations (Bachtrog *et al*. 2014). If sexual antagonism is a main driver of their evolution, sexually antagonistic selection must differ between these groups to generate the variation in sex determination (SD) systems, but both the sources and effects of differences in sex-specific selection pressures according to ecology are still generally unknown ((Abbott *et al*. 2017); but see (Connallon and Clark 2014; Connallon 2015)). Other hypotheses for the drivers of sex determination switches or sex chromosome evolution- including neutral processes, and sex-ratio selection due to selfish genetic elements (Beukeboom and Perrin 2014)- do not act based on ecological differences between species or populations. However, heterogeneity within lineages is itself a useful tool for understanding such questions (Bachtrog *et al*. 2014). Species with labile sex determination are ideal for comparative studies, both where sex chromosomes are young and have not yet spread between populations and where sex determination has evolved differentially among populations according to differing selection pressures (Furman *et al*. 2020). By utilising such systems, theoretical ideas on SD switches and drivers of early sex chromosome evolution and the role of ecology in these processes can be tested.

While differences in selection between sexes can lead to the evolution of sex chromosomes and sexual dimorphism, differences in sex-specific selection between populations can drive the evolution of sex chromosome differences between the populations (Abbott *et al*. 2017; Bracewell *et al*. 2017; Wright *et al*. 2017; Lasne *et al*. 2018). Similarly, differences in natural selection between populations can drive adaptive divergence and the build-up of reproductive isolation (Barton and Hewitt 1989; Coyne and Orr 2004; Butlin 2010; Johannesson *et al*. 2020). Indeed, it is often the case that the same selection pressures can drive differentiation between sexes and populations simultaneously, highlighting the non-independence of the processes and the importance of incorporating ecology into the study of sex chromosome evolution (e.g., Puixeu *et al*. 2019). Further, in both instances, the suppression of recombination between relevant loci aids the build-up of differentiation between sexes/populations, and chromosomal inversions are often a key mechanism to suppress recombination (Charlesworth 1991; Kirkpatrick and Barton 2006; Jackson *et al*. 2016; Kirkpatrick 2017; Olito and Abbott 2023). It is possible, therefore, that the same inversions may play a role in both cases.

Hybrid zones are ideal systems for investigating links between population divergence, selection, genetics, and the environment (Barton and Hewitt 1985; Barton and Hewitt 1989). The coincidence of genetic or phenotypic clines with clines in environmental factors can help to identify selection pressures (Barton and Hewitt 1985; Barton and Hewitt 1989). Examining

multiple equivalent hybrid zones between populations of a species can further enhance studies as the replication can help to verify that patterns are likely the result of systematic effects rather than unique outcomes (Zieliński *et al*. 2019; Westram *et al*. 2021). Both similarities and differences between replicates can also help to identify key selection pressures and regions of the genome involved in divergence (Westram *et al*. 2021). Sharing between populations highlights importance, while differences may help disentangle factors that often coexist making it difficult to assess their individual importance. The level of similarity of sex determination systems across multiple populations of a species can be informative about the age, origin and spread of the system in addition to environment- and sex-specific selection pressures that may have driven their evolution (Furman *et al*. 2020).

The intertidal snail *Littorina saxatilis* makes an ideal system for this approach (Johannesson *et al*. 2020). It can be found in varying habitats along rocky shores, where local adaptation has produced distinct ecotypes (Johannesson *et al*. 2010). In Sweden, where our study is based, two main ecotypes are present that differ phenotypically. The Crab ecotype is adapted to withstand predation from crabs in boulder fields; they are large, thick-shelled, with a relatively small aperture (Boulding and Van Alstyne 1993; Johannesson *et al*. 2010; Butlin *et al*. 2014). The Wave ecotype inhabits rocky cliffs and has adapted to avoid being swept away by wave action; individuals are small and thin-shelled to enable sheltering in crevices, with a relatively large aperture to maximise the area of the foot anchoring to the rock (Boulding and Van Alstyne 1993; Johannesson *et al*. 2010; Butlin *et al*. 2014). Hybrid zones are formed at habitat transitions (Panova *et al*. 2006; Hollander *et al*. 2015), where ecotypes interbreed despite some assortative mating (Johannesson *et al*. 1995; Hollander *et al*. 2005; Perini *et al*. 2020). Transitions between habitats and ecotypes occur over very small scales (only tens of metres) (Grahame *et al*. 2006); this is facilitated by the restricted lifetime dispersal of *L. saxatilis* as a result of ovoviviparity (Reid 1996). As a result, multiple separate hybrid zones, which have evolved in parallel, can be found within small areas, providing easy replication (Panova *et al*. 2006; Johannesson *et al*. 2010; Butlin *et al*. 2014; Westram *et al*. 2021).

L. saxatilis has been well-utilised for studying local adaptation with gene flow. In a set of seven Swedish hybrid zones, numerous outlier SNPs have been identified that differ clinally between the ecotypes and many of them are shared between hybrid zones (Westram *et al*. 2018; Westram *et al*. 2021). In addition, polymorphic inversions have been identified on multiple linkage groups in the same hybrid zones (Faria *et al*. 2019; Westram *et al*. 2021). Frequency differences between the ecotypes are present for some inversions and are associated with adaptive traits and outlier SNPs, suggesting a role in local adaptation (Westram *et al*. 2018; Faria *et al*. 2019; Morales *et al*. 2019; Koch *et al*. 2021; Westram *et al*. 2021). This is supported by the association of cline centres with habitat transitions. Moreover, these associations are present for some inversions across multiple hybrid zones (Westram *et al*. 2021; Koch *et al*. 2022). At one Swedish site, three of four inversions that have been identified along Linkage Group 12 (LG12) show both sex- and ecotype-associated patterns in frequency (Hearn *et al*. 2022). They provide evidence for a ZW genetic sex-determination system in this population, but this is detectable only in the Crab ecotype. Multiple traits have been shown to be sexually dimorphic (e.g., Fretter and Graham 1962; Larsson *et al*. 2020), and a recent study has additionally demonstrated an influence of an LG12 inversion on some of these traits (Koch *et al*. 2021; Koch *et al*. 2022). The ecotype-specific sex-inversion associations indicate an interaction between sex-specific selection and locally-adaptive selection and suggest the dual

role of these inversions in sex chromosome evolution and adaptive divergence between the ecotypes (Hearn *et al*. 2022).

This sex-determination system has only been identified in a single population of *L. saxatilis* at present. Therefore, this study aimed to test for a genetic sex-determination system and for association of sex with inversions across six more hybrid zones in Sweden, using the same replicates as in Westram *et al*. (2021). Comparison between these sites and the population studied by Hearn *et al*. (2022) provides insight into the relationships between sex-determination systems, inversions, and ecotype divergence, and the selective pressures underlying them.

3.3 Methods

This study examines six hybrid zones on three islands, utilising a dataset previously published in Westram *et al*. (2021). The sites are highly similar to each other in phenotypes and environmental conditions and show a lack of genomic differentiation, although exposure (and therefore wave action) does differ between Wave habitats between and across sites. For full details of sampling methodology and data generation, see Westram *et al*. (2021); they are summarised below. Additionally, analyses in this study follow similar methods to those employed in Hearn *et al*. (2022), which were performed on the related hybrid zone (ANG: Ångklåvebukten) dataset from Westram *et al*. (2018). The three islands in this study were all compared to the findings in ANG for all analyses. All analyses were implemented in R (version 4.0.0; R Core Team 2020), using the packages DPLYR (version 1.0.5; Wickham *et al*. 2021) and GGPLOT2 (version 3.3.0; Wickham 2016) unless otherwise stated.

Sampling and genotyping

The three islands in this study are all located on the Swedish west coast close to ANG: Ramsö (58°49’27.8”N, 11°03’45.3”E), Inre Arsklovet (58°50’00.5”N, 11°08’19.6”E) and Yttre Arsklovet (58°49’51.3”N, 11°07’59.0”E), hereafter named CZA, CZB and CZD, respectively (Figure 1). The three islands and ANG are all less than 7km apart (Figure 1). In a single bay on each island, a transect was sampled (about 600 snails) that spanned two hybrid zones: from rocky cliffs to boulder field to rocky cliffs. The two areas of Wave habitat- rocky cliffs- are referred to as the ‘left-hand’ and ‘right-hand’ Wave habitats according to their position to the left or right of the Crab habitat (boulder field) when looking at the shore from the sea (Figure 1C). These names were used for these habitats in Westram *et al.* (2021), so are used again here for consistency and clarity. The three-dimensional position of each snail on the shore was recorded and used to calculate a one-dimensional path across the shore on which all snails were included. All snails were phenotyped for size, shape, and sex.

Of these, 382-384 snails per transect were genotyped. DNA was extracted using a protocol from Panova *et al*. (2016) and genotyped through capture sequencing using 40,000 randomly distributed probes (120bp length), the same as those in Westram *et al*. (2018). Read mapping, quality control and filtering are described in Westram *et al*. (2021). Only snails for which there were genotype, sex, and transect position information available were retained for use. This left 363 snails for CZA, 381 for CZB, and 369 for CZD.

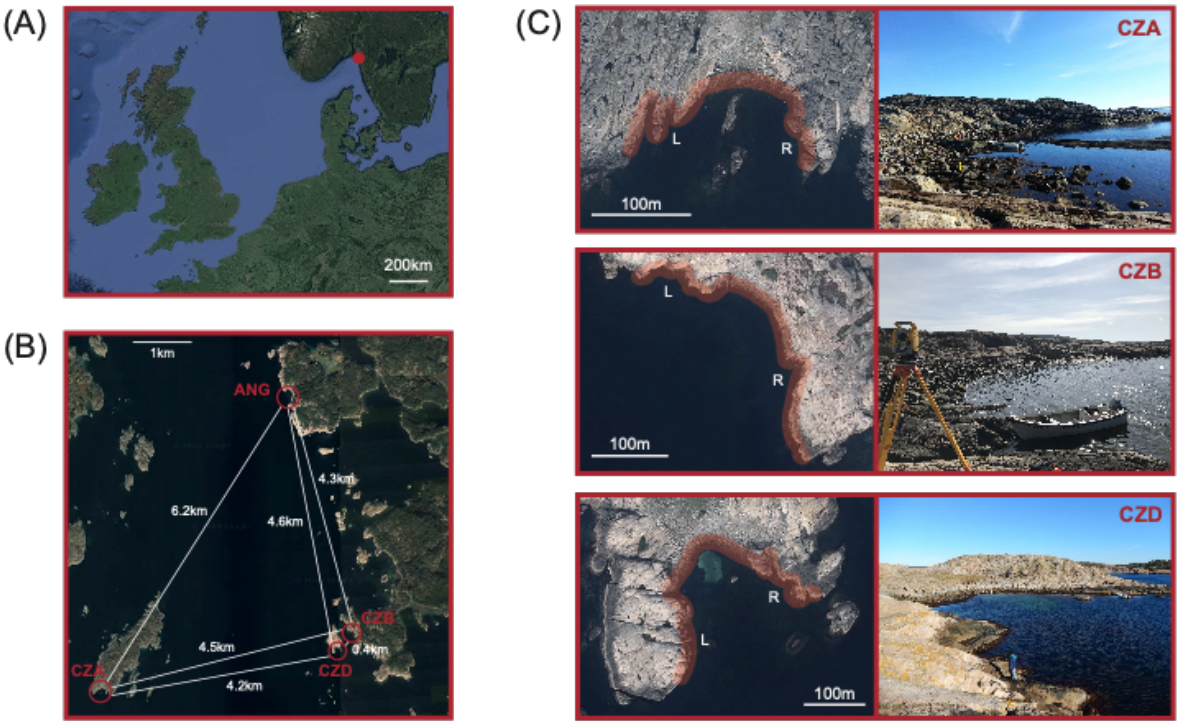

Figure 1. (A) The location on the west coast of Sweden of the sampling area, marked with a red dot. **(B)** Map of the three islands sampled: Ramsö (CZA), Inre Arsklovet (CZB) and Yttre Arsklovet (CZD), and their location in relation to the site studied in Hearn *et al*. (2020) (ANG). Distance between all sites is shown. The bay sampled on each island is circled in red. **(C)** Arial view and photographs of the bay studied on each island. The transects in CZA, CZB and CZD all cross two transitions, from Wave (rocky headland) to Crab (boulder field) to Wave (rocky headland), and are approximately highlighted by an orange curve in the arial images. The two areas of Wave habitat are referred to as left and right according to their position relative to the Crab habitat, when looking inland from the sea (labelled with 'L' and 'R' on arial view). The photographs are each taken from a position on the left-hand Wave habitat and look across the transect towards the right-hand Wave habitat. Satellite images taken from Google Maps.

Ecotype classification

Ecotype grouping was required for some analyses; to classify snails by ecotype, the snail position on the transect was used in conjunction with the main environmental transition (Westram *et al*. 2021) for each hybrid zone (Supplementary Table 1). Snails within 10m of each transition were classified as hybrids (Supplementary Tables 1-2). Hybrids were removed for some analyses; this left 316 snails at CZA (171 females and 145 males), 318 at CZB (176 females and 142 males), and 315 at CZD (170 females and 145 males) (Supplementary Table 2).

Heterozygosity for detection of sex-associated regions

SNPs in a sex-determining region (i.e., a region of restricted recombination that includes a sex-determining locus) are expected to reflect the genotype of the sex-determining locus that

they are linked to; SNPs outside this region, but still on sex chromosomes, are not expected to show significant sex differences in genotype frequencies (Pucholt *et al*. 2017; Palmer *et al*. 2019). Therefore, heterozygote frequencies were compared between the sexes within each ecotype. In ANG, different linkage groups were found to show sex-association in each ecotype, strongly for LG12 in Crab and weakly for LG5 in Wave (Hearn *et al*. 2022), despite close proximity on the shore; therefore, all linkage groups were tested in the three populations in this study as other regions of the genome may be sex-linked in these populations. In total, 40,549, 70,738, and 80,937 SNPs across 17 linkage groups were tested in CZA, CZB and CZD, respectively.

For each linkage group, snails were separated into sex and ecotype groups (Supplementary Table 2), and genotype frequencies were calculated per SNP. The heterozygosity of SNPs was compared between males and females in each ecotype. Sex differences in heterozygosity per SNP were quantified by calculating residuals from the null expectation of no sex difference (i.e., 1:1 relationship) as male heterozygosity minus female heterozygosity. As in ANG, possible signals in Wave were less distinctive than in Crab so the 1% of SNPs with the most negative residuals in Wave was calculated and their distribution across the linkage groups checked. This association was tested for significance using chi-square tests.

Any linkage group that showed possible signals of sex-associated SNPs was examined further. The heterozygosity residuals of SNPs were plotted against the genetic map (from Westram *et al*. 2018) for that linkage group to identify the locations of any sex-determining regions.

Inversion detection

Inversions are often found on sex chromosomes and are a possible mechanism for generating a sex-determining region through recombination suppression (Charlesworth 1991; Wright *et al*. 2016). Putative inversions were identified on a chromosome with a sex-determining locus in *L. saxatilis* at the nearby ANG site (Hearn *et al*. 2022), on the linkage group (LG12) that displayed the strongest patterns of sex association. Therefore, inversion detection was carried out independently on LG12 in CZA, CZB and CZD to test whether the same inversions are present here as in ANG. The same inversion detection methodology was employed here as in Hearn *et al*. (2022), similar to that in Faria *et al*. (2019).

All females were included in the first step of the analysis for each island. A matrix of pairwise LD (r^2) values for all SNPs was calculated using the package GENETICS (version 1.3.8.1.2; Warnes *et al*. 2019). Following this, the LD matrix was examined with the package LDNA (version 0.6.4; Kemppainen 2014) to identify clusters of SNPs under higher LD than the background level. Outlier clusters of interest were identified for downstream analysis through manipulation of two LDNA parameters: $|E|_{min}$ and φ (see Hearn *et al*. (2022) for full details of LDNA analysis and selection criteria for outlier clusters). The distribution of SNPs in each outlier cluster along the linkage group genetic map was also examined.

LD clusters of interest were investigated using principal component analysis (PCA) to identify whether they were likely to be signals of inversions. A PCA of an inversion region is expected to show distinct clusters of individuals, grouped by their inversion genotype. A single inversion would show three groups, the two homozygote groups and the heterozygous group, if all three

genotypes are present in that population. PCA was carried out for each outlier cluster, using all SNPs within the region of LG12 that the cluster covered and all individuals (males and females). The packages HMISC (version 4.4.0; Harrell Jr and Others 2020) and ADEGENET (version 2.1.3; Jombart 2008; Jombart and Ahmed 2011) were used for the PCA analyses.

Analysis of heterozygosity (above section) revealed a signal of sex-association in the Wave ecotype in CZA, on LG5 rather than LG12; analogous to that seen in ANG by Hearn *et al*. (2022). Therefore, LG5 was also tested for the presence of inversions in ANG as well as CZA. As the sex-associated SNPs were spread across almost the entire linkage group, the LDNA step was excluded and PCA was carried out on the whole of LG5. The analysis used the 7282 SNPs present on LG5 in ANG and 2679 in CZA.

Cline analysis of inversions using splines

Splines were fitted to the putative LG12 and LG5 inversion genotypes identified in the previous section to clarify patterns in arrangement frequency fluctuations across the transect from Crab to Wave. Differences in those patterns between sexes were also tested by fitting splines separately to arrangement frequency in each sex. In each case for LG12, the inversion arrangement more frequent in Crab than Wave in females was used for spline fitting; this arrangement was named the reference (R) and the other the alternate (A). To visualise splines, the frequency of the R arrangement in each sex across the transect was calculated for each inversion on each island using sliding windows of 25 snails, moving by five snails between windows. 'Arrangement' frequencies for LG5 were also calculated for visualisation in the same manner, treating the two PCA groups identified on LG5 in ANG and CZA as homozygote RR and heterozygote RA genotypes of an inversion.

The glm function in the R package rms (version 6.2.0; Harrell Jr 2021) was used to fit splines. Splines were fitted to individual genotypes coded as binomial data, to each sex separately and to the data of both sexes together. For each inversion at each site, splines were fitted with varying numbers of knots between three and ten and the AIC values extracted. The AIC values for male and female splines were added at each number of knots to compare against the AIC value for the spline fit to both sexes together. Lower AIC values for the sum of male and female splines than the combined spline at all knots for an inversion indicated that there was a sex difference in inversion clines, whereas the vice versa indicated that inversion frequencies did not differ between the sexes. A sex difference was equivocal if the spline(s) with the lower AIC value varied between combined or separate sexes at each number of knots.

Allele frequency differences of inversions and collinear SNPs between ecotypes

To provide some context to the inversion arrangement frequency differences between the ecotypes, allele frequency differences (AFDs) were calculated as an indicator of background genetic differentiation. AFDs were calculated for all collinear SNPs in the genome (excluding LG12 and inversion locations from Faria *et al.* (2019)). This included a total of 30,412 SNPs for CZA, 52,988 for CZB, and 60,264 for CZD. Separate AFDs were calculated for each sex, and the two Wave habitats were kept separate, giving four sets of AFDs per site (Crab vs left-hand Wave and Crab vs right-hand Wave for each sex). Twenty snails of each sex were used for the calculation of allele frequencies; for the Wave habitats, these were the snails at the most distant ends of the transect (furthest from the Crab habitat) and for the Crab habitat, these were the most central Crab snails by distance from the habitat transitions (furthest from

Wave habitats). The absolute difference in allele frequency between Crab and Wave was taken as the AFD as the direction of difference was not important here. The 99th percentile for AFD was calculated for each set of comparisons. AFDs were also calculated for the LG12 inversions, using arrangement frequencies. These were then compared to the distributions of AFDs of collinear SNPs.

Associations between inversions and between inversions and sex

Within each population, the association between genotypes at pairs of inversions were tested separately for each sex and ecotype using chi-square contingency tests (using packages ZOO (version 1.8.8; Zeileis and Grothendieck 2005) and TIDYQUANT (version 1.0.3; Dancho 2021)). The strength of association in each case was quantified using squared correlation coefficients. The association between inversion genotypes and sex was also assessed in the same way for each sex in each population.

3.4 Results

Patterns of heterozygosity in the Crab ecotype

The heterozygosity of SNPs was compared between the sexes for all linkage groups within each ecotype. Sex differences in allele and genotype frequencies of SNPs are expected when they are linked to a sex-determining locus (Pucholt *et al*. 2017; Palmer *et al*. 2019); specifically, the same pattern of heterozygosity as the sex-determining locus is expected. At ANG, the Crab ecotype exhibited a female-heterogametic sex-determining region in a central region of LG12 (Hearn *et al*. 2022). If the Crab ecotypes at the CZ sites share a sex-determination system with ANG, similar patterns are expected on LG12.

This was indeed displayed in the heterozygosity analysis. In all three islands, LG12 alone showed signals of sex-linkage (Supplementary Figure 1). As in ANG, heterozygosity was skewed towards females, such that a high proportion of females were heterozygous at SNPs while the proportion of heterozygous males was low (Supplementary Figure 1). However, the strength of sex-association varied between islands: none showed as strong an association as ANG. Residuals (male heterozygosity minus female heterozygosity) reached a maximum of -0.72 in CZA, -0.63 in CZB and -0.58 in CZD (Figure 2a). In contrast, residuals in ANG reached -0.96, almost complete sex-association.

The distribution of sex-associated SNPs along the LG12 genetic map was examined. In CZA, those SNPs were positioned in precisely the same central region of LG12 as in ANG, between 33cM and 48.7cM (Figure 2a). This region is spanned by two inversions in ANG (LGC12.2 and LGC12.3). The sex-associated SNPs also clustered in the centre of LG12 in CZB and CZD; however, here they covered only a smaller region from 33cM to 43.8cM (Figure 2a). These are the breakpoints of the LGC12.2 inversion in ANG. Unlike ANG, none of the CZ sites showed any moderately sex-associated SNPs (medium residuals) at the end of the linkage group from 48.7cM to the end (Figure 2a).

Putative inversions in Crab on a chromosome implicated in sex

Inversions frequently evolve on sex chromosomes; they may be part of the mechanism by which the sex chromosomes themselves evolve (Charlesworth 1991; Wright *et al*. 2016), or

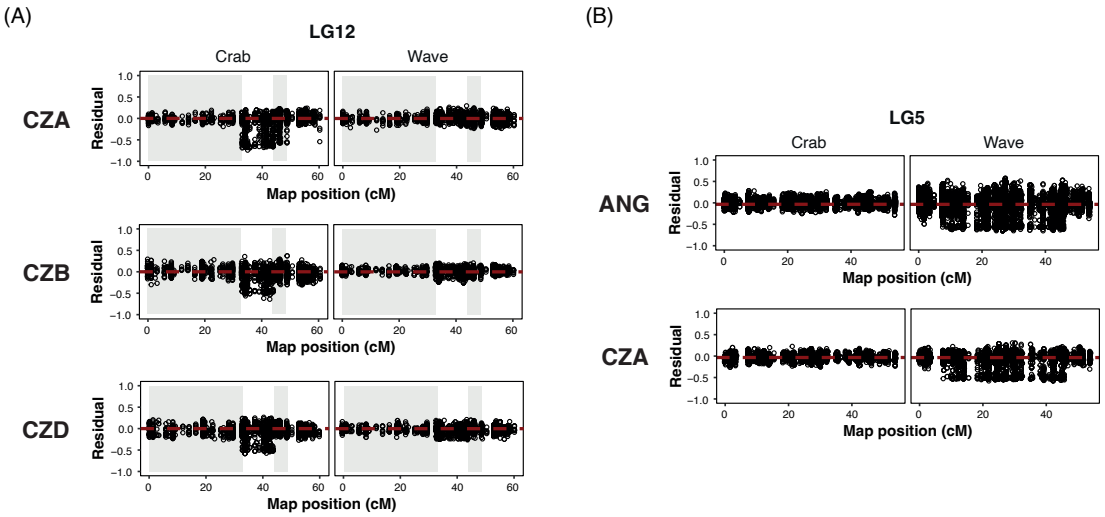

Figure 2. The distribution along their genetic maps of SNPs across **A)** LG12 for the three CZ sites and **B)** across LG5 for ANG and CZA, the two sites with signals of sex-association on LG5. Sex association is signified through residuals, which quantify the difference in heterozygosity between the sexes (calculated as female heterozygosity minus male heterozygosity). Larger residuals represent a stronger sex association, where the difference in the proportion of heterozygotes between the sexes is larger and further from the neutral expectation of equal proportions between the sexes (Supplementary Figure 1). Alternating grey shaded and white non-shaded areas of the background for LG12 represent the position of the four LG12 inversions.

may appear on established sex chromosomes (Sun *et al*. 2017; Furman *et al*. 2020). Hearn *et al*. (2022) identified a series of four putative inversions that span the chromosome with a sex-determining locus in Crab (LG12) of *L. saxatilis* at ANG. Here, we independently tested for presence of inversions on the same chromosome pair identified in the Crab ecotype at each CZ site (identified above as LG12 in all sites). It is probable that similar inversions may be present in these sites as at ANG since the patterns of sex-association are alike.

Inversion detection was carried out through investigation of LD clusters in females followed by PCA of both sexes. In each site, three (CZB and CZD) or four (CZA) main LD clusters of interest were identified for further analysis. (See Supplementary Results for full details). SNPs in each of the clusters were located in distinct regions of LG12, and in all cases, the cluster position at least approximately matched the location of one of the ANG inversions. Thus there was a high level of similarity between the four sites. Clusters spanning 33-43cM and 48-60cM were found in all three CZ sites, matching the positions of putative inversions LGC12.2 and LGC12.4. A cluster also covered the first half of LG12 in all sites, albeit with some variation in the end point: the cluster ended at 33cM in CZB and CZD, as in ANG's LGC12.1, but reached 35cM in CZA. Finally, the region occupied by LGC12.3 in ANG (43-48cM) was represented by a cluster in CZA but this was not present in CZB or CZD. Therefore, based on LD clusters, it would appear that three of the four ANG inversions are shared between all of the sites in this study, while the fourth is restricted to only two sites. This was investigated further using PCA to confirm the presence of inversions in these regions.

PCA was carried out on the regions of LG12 covered by clusters of interest, and additionally on the LG12 inversion regions where they varied from the cluster positions. Distinct clusters of individuals grouped according to inversion genotype are expected on a PCA of SNPs in an inversion region. In all cases where the cluster region matched an ANG inversion region (i.e. all clusters other than the first cluster on CZA), three distinct groups along PC1 without intermediates were visible (Supplementary Figure 2), indicative of likely inversions. The region 0-35cM in CZA produced a less distinct PCA with three to five groups defined by both PC1 and PC2, and with intermediate individuals (Supplementary Figure 2). In contrast, PCA of the LGC12.1 region (0-33cM) in CZA gave three clear groups along PC1 (Supplementary Figure 2), suggesting that the inversion in this region does share the ANG breakpoints but some SNPs from the neighbouring inversion (LGC12.2) were captured in this cluster. In CZA and CZB, a small number of snails (four and one, respectively) were always located at extreme positions on the PCAs a long distance from all other snails. These snails had a high percentage of missing data so were removed, and repetition of the PCAs solidified the clustering in all cases. PCA was additionally carried out on the LGC12.3 inversion region in CZB and CZD despite no LD clusters being identified in this region, to corroborate that no polymorphic inversion was present in each location. As expected from the lack of LD cluster, individuals on the PCA did not cluster into separate groups and instead formed a continuous cloud of points in both cases (Supplementary Figure 2). Overall, PCA supports the presence of same four inversions identified in ANG in all three CZ sites with the exception of LGC12.3 in CZB and CZD. From this point on, the inversions in CZ sites will be named as they are in ANG: LGC12.1, LGC12.2, LGC12.3 and LGC12.4.

Sex and ecotype differences in inversion frequencies

Genotype and arrangement frequencies were calculated for each inversion in each sex along the transects, and a variety of spline models fitted. Sex-linked inversions will show sex differences in frequency and divergently selected arrangements will differ in frequency clinally between the ecotypes. Clines were expected in all inversions in at least one sex, as in ANG, and sex differences in Crab expected in inversions covering those regions that showed sex-association in earlier analyses (i.e., LGC12.2 in all CZ sites and additionally LGC12.3 in CZA).

Frequency differences between ecotypes and sexes were visible in most cases (Figure 3), and comparison of splines for each inversion suggested which of these were significant (Table 1). As two hybrid zones were spanned at each CZ site rather than one as in ANG, the distribution of individuals along each transect was more sparse than at ANG (by roughly 50%). As a result, windows of snails covered much wider regions of the transect, and therefore variable environments, and so in some cases arrangement frequency estimates were very variable between windows.

Patterns of heterozygosity between the sexes in the region spanned by LGC12.2 were replicated in the CZ sites so LGC12.2 was expected to show sex-linked clines comparable to those at ANG for all CZ sites. In the three sites, distinct sex differences in arrangement frequency were visible in the Crab ecotype (and in a small gully at CZA with Crab-like habitat; Figure 3). The arrangement remained close to a frequency of 0.5 in females as it did in ANG; however, the frequency in males varied between sites, between almost fixation in CZA (as in ANG) and more intermediate (0.85) in CZB and CZD. In all sites, some sex difference in frequency persisted at one of the Wave ends of the transect- at the right side in CZA and CZB

and the left in CZD. Male and female frequencies roughly converged in the other Wave end in each case to an intermediate frequency. Sex differences were confirmed by the spline fits for the three sites, where the AIC for the sum of male and female splines was lower than the spline of the combined data at all numbers of knots (Table 1). Sex-specific clines were expected for LGC12.3 in CZA. Visually, changes in arrangement frequency followed that of the inversion in ANG (Figure 3). As with LGC12.2, the female frequency in Crab was close to 0.5 with Crab males almost fixed for one arrangement. Female arrangement frequency dropped to that of males in both Wave regions of the transect, although it regained sex difference in the Crab-like gully (Figure 3). Spline fits confirmed the clear sex difference in frequency across the transect as, at all numbers of knots, the AIC value was lower for the sum of separate male and female splines than the spline fitted to the combined data (Table 1).

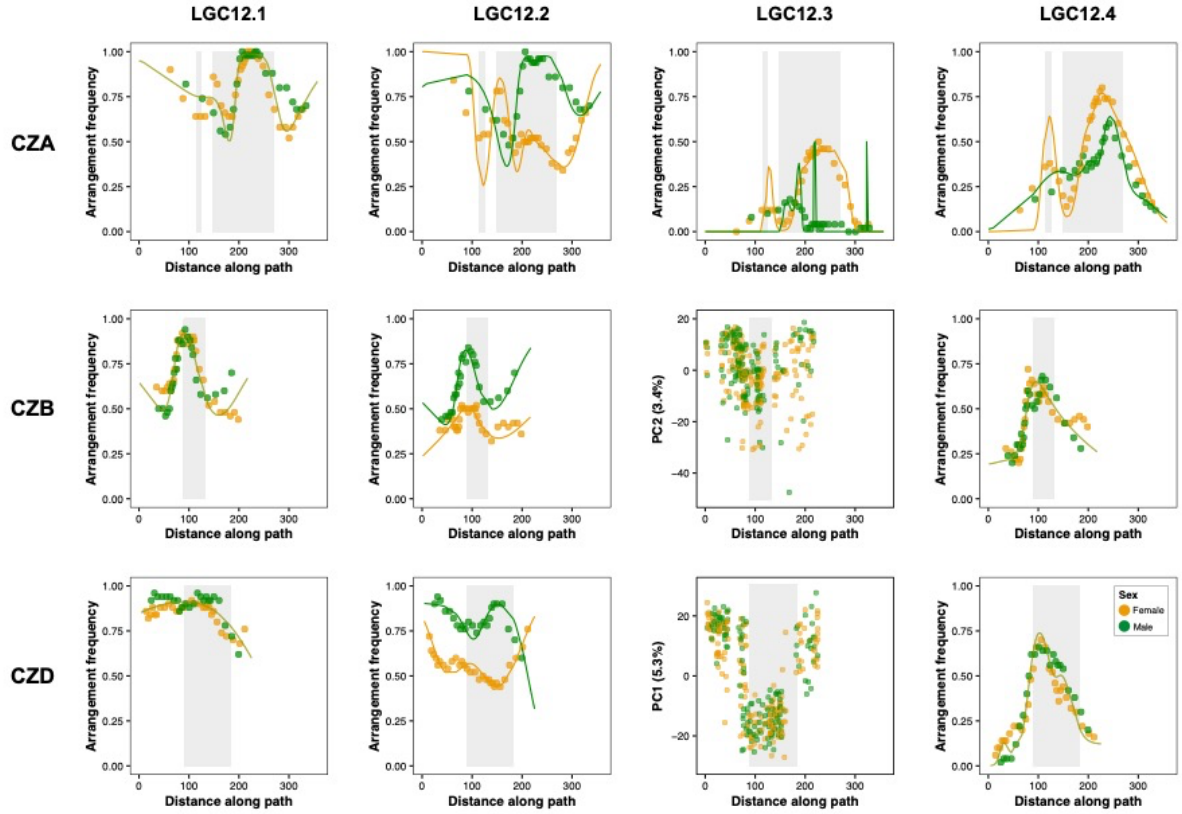

Figure 3. The frequency of LG12 inversion arrangements in overlapping, sliding windows across the transect (Wave to Crab to Wave) in each sex at the three CZ sites (points), and the best-fitting spline models for each of these (lines). A combined spline was better fitting than separate male and female splines for LGC12.1 and LGC12.4 in CZB and LGC12.4 in CZD, and there was an equivocal sex difference for LGC12.1 in CZA and CZD so the combined spline is also shown in those cases. For the LGC12.3 region in CZB and CZD where no inversion was detected, the PC axis that spread individuals the most was plotted along the transect. Grey shading represents areas of Crab habitat, as determined by the distribution of PC1 along the transects from a PCA of environmental variables. The smaller shaded area in CZA represents a small gully within the left-hand Wave area that has a Crab-like habitat.

Table 1. AIC values for splines fitted to the distribution of inversion arrangement frequencies along the transect at each site. For each inversion at each site, splines with 3-10 knots were fit separately to male and female frequencies (and the AIC summed) and to the male and female data together. For each number of knots for each site and inversion, the AIC value that is lower out of male+female models or combined model is highlighted by shading, with the other also highlighted by paler shading if the difference between the pair of AICs is two or smaller. For each inversion at each site, the spline with the lowest AIC (i.e. the best fitting) out of the shaded splines is highlighted in bold.

Site	Inversion	Knots	Female	Male	Female + male	Combined
CZA	LGC12.1	3	315.1826	251.3953	566.5779	565.0755
		4	301.4874	240.1129	541.6003	543.4582
		5	277.0038	216.5685	493.5723	502.946
		6	253.6171	214.5865	468.2036	463.3455
		7	256.9931	205.3087	462.3018	457.3204
		8	244.6399	199.8069	444.4468	457.5892
		9	321.335	200.9229	432.2579	458.492
		10	226.0029	205.1439	**431.1468**	454.3304
	LGC12.2	3	342.9113	270.7855	613.6968	668.0848
		4	343.0479	258.0871	601.135	664.5856
		5	337.1973	239.7671	576.9644	653.1848
		6	336.3102	240.4471	576.7573	645.3433
		7	334.1325	237.4807	571.6132	647.09
		8	329.1387	228.7536	557.8923	649.4892
		9	316.8426	228.9202	**545.7628**	650.2452
		10	315.0979	235.1284	550.2263	642.8359
	LGC12.3	3	212.0579	80.03215	292.09005	346.618
		4	204.9988	71.51571	276.51451	347.3245
		5	206.5626	71.58353	278.14613	349.1186
		6	207.8882	72.89568	280.78388	349.7153
		7	202.4259	72.23598	274.66188	350.9126
		8	200.8568	61.18744	262.04424	351.9607
		9	197.4595	64.30332	261.76282	351.4625
		10	196.7887	54.69595	**251.48465**	349.1835
	LGC12.4	3	327.7246	277.0981	604.8227	609.8529
		4	316.7421	276.548	593.2901	598.341
		5	318.9914	278.9463	597.9377	600.8665
		6	310.2279	276.6373	586.8652	597.781
		7	305.1355	277.7747	582.9102	593.3353
		8	303.0255	279.8421	582.8676	592.4927
		9	297.1199	280.4459	**577.5658**	591.7234
		10	296.2784	282.0928	578.3712	588.1521
CZB	LGC12.1	3	352.2887	306.6105	658.8992	660.6171
		4	354.345	308.7424	663.0874	663.4432
		5	333.6299	284.1946	**617.8245**	619.153
		6	336.8515	285.283	622.1345	621.8965
		7	337.3776	288.3383	625.7159	622.0647
		8	338.2344	287.9101	626.1445	622.1371
		9	338.9078	288.5007	627.4085	623.7052

		10	341.6358	290.2418	631.8776	625.2076
	LGC12.2	3	336.2469	312.9626	649.2095	675.3592
		4	335.96	314.2136	650.1736	672.8145
		5	336.7761	301.3852	**638.1613**	664.8622
		6	338.7941	302.2498	641.0445	666.8765
		7	339.8232	305.3593	645.1825	667.9149
		8	341.0187	304.888	645.9067	668.1929
		9	342.1111	306.3039	648.415	669.2865
		10	344.744	307.8599	652.6039	669.8619
	LGC12.4	3	373.1149	294.5702	667.6851	664.1207
		4	375.5671	294.7865	670.3536	666.7208
		5	373.2353	294.1342	667.3695	**658.4994**
		6	370.373	296.2546	666.6276	659.7727
		7	374.3227	298.0263	672.349	661.3034
		8	372.5451	298.5721	671.1172	659.4947
		9	370.3778	300.3927	670.7705	659.4341
		10	370.6803	301.3663	672.0466	658.9333
CZD	LGC12.1	3	238.8861	165.2875	**404.1736**	407.6316
		4	240.4658	164.494	404.9598	409.7489
		5	241.2834	164.494	405.9425	411.7399
		6	240.5106	164.5578	405.0684	409.6242
		7	241.7528	166.3947	408.1475	410.7154
		8	243.3588	168.0582	411.417	411.4679
		9	245.5346	167.9456	413.4802	412.5571
		10	247.5001	166.6652	414.1653	413.3257
	LGC12.2	3	306.0711	223.7645	529.8356	579.4988
		4	306.2719	218.2478	524.6197	581.3507
		5	304.6501	216.0173	**520.6674**	583.3328
		6	306.1965	215.5833	521.7798	585.2777
		7	307.5675	215.621	523.1885	587.145
		8	309.454	216.4568	525.9108	588.5839
		9	311.4981	216.9668	528.4649	590.0796
		10	313.4953	218.148	531.6433	592.1075
	LGC12.4	3	272.9462	214.7483	487.6945	488.4363
		4	275.5538	216.1829	491.7367	491.736
		5	274.8365	217.7142	492.5507	486.4522
		6	276.7498	215.9703	492.7201	491.2492
		7	270.6555	218.6157	489.2712	486.8975
		8	272.3965	218.6891	491.0856	485.0436
		9	271.4479	220.8117	492.2596	**484.7544**
		10	272.5854	222.2983	494.8837	485.8814

Although no inversion was detected in the LGC12.3 region in CZB and CZD, the scores from the PCA axis that spread individuals the most (PC2 for CZB; PC1 for CZD) were plotted against the transect distance. PC2 did not distinguish the ecotypes in CZB but PC1 varied clinally along the transect to separate the ecotypes at CZD (Figure 3). There was no overlap between the highest loading SNPs to PC1 in CZD and either PC1 or PC2 in CZA, although the proportion of SNPs in both datasets was relatively low (1576 shared out of a total 4064 in CZA and 2217 in CZD). No sex differences were present in either CZB or CZD for the LGC12.3 region.

Expectations for sex differences in the frequency of LGC12.4 differed between the CZ sites according to the presence or absence of the LGC12.3 inversion. In ANG, it was hypothesised that the small sex difference present in LGC12.4 was due to LD with the sex-linked region (LGC12.2 and LGC12.3). As LGC12.3 is absent in CZB and CZD, no sex differences were expected as LGC12.4 was unlikely to be in LD with any sex-linked region. In contrast, sex differences were anticipated in CZA where LGC12.3 is present. Frequencies in CZA did follow a similar clinal pattern to ANG, with an intermediate frequency and a small sex difference of around 0.2 in Crab (smaller than that at ANG) and in the Crab-like gully, falling to a low frequency (around 0.1) in both sexes in both the left and right Wave regions (Figure 3). The sex difference was confirmed once more by the spline fits (Table 1). Arrangement frequencies followed the predicted cline in CZB and CZD, with parameters much alike to ANG and CZA aside from the lack of sex difference in Crab (Figure 3). Both males and females had an arrangement frequency of around 0.65-0.7 in Crab- intermediate to the frequencies in the two sexes in CZA. Spline fitting confirmed no sex difference at either CZB or CZD; for the best fitting splines at each site, the AIC values were lower for the combined splines than the sum of male and female splines (Table 1).

In ANG, the only inversion that did not show any sex-association was LGC12.1. Clinal variation was present, however, with one arrangement fixed in Crab becoming polymorphic in Wave. Both CZA and CZB exhibited similar patterns in arrangement frequency to this inversion (Figure 3). CZA was the most alike to ANG; the arrangement was essentially fixed in Crab and fell to a frequency of around 0.7 in Wave. It was equivocal whether the separate male and female or combined splines were the best fit (Table 1), meaning there was no clear sex difference. The combined spline not fitting best at all numbers of knots was likely driven by the variability in frequency in parts of the Wave regions which was slightly different between the sexes at various points, although the frequency did not differ between sexes in Crab. Frequencies in both ecotypes were lower in CZB than CZA and ANG but showed a comparable drop of around 0.3 in frequency (from 0.9 in Crab to 0.6 in Wave) (Figure 3). Once more the best fitting spline model varied at different numbers of knots so did not reveal any clear sex difference (Table 1). Finally, arrangement frequencies did not show a clear cline in CZD (Figure 3). Sexes did not differ in arrangement frequency and the frequency began high at 0.9 in Crab similarly to the other three sites. The frequency did drop somewhat to nearer 0.7 in the right-hand Wave region but remained as high as in Crab in the left-hand Wave region. Spline fitting did not detect a sex difference, as in the other three sites (Table 1).

Overall, clines were generally replicated across the four sites with some population-specific modifications. One parameter that varied consistently between sites for all inversions was the ‘Crab zone’ of arrangement frequency, i.e. the region in between the left and right cline centres where the frequencies were Crab-like, and its position in relation to the main environmental transition for each hybrid zone (Figure 3). In ANG, clines were displaced into the Wave habitat for all LG12 inversions. This did not appear to be the case for several of the transitions in the other sites. Cline centres were clearly within the Crab environment of the shore (in between the left and right environmental transitions) for all left-hand sides of CZA clines and right cline centres were relatively close to the environmental transition. The overall zone of Crab frequency was relatively narrow in relation to the Crab environment and located in between the centre of the Crab habitat (halfway between the two transitions) and the right-hand environmental transition. Clines were also shifted in CZB but to the left- the less exposed side of the shore- and to a greater extent. Right-hand cline centres were shifted left into the Crab

Table 2. The correlation (r^2) and significance (p-value; in brackets) of associations among genotypes at pairs of inversions for each sex and ecotype group at each site. The final column gives the correlation and significance of the association of inversion genotype with sex for each inversion in each ecotype at each site.

			LGC12.2		LGC12.3		LGC12.4		Sex
			F	M	F	M	F	M	
CZA	LGC12.1	C	**0.3884** (5.20x10^{-9})	**0.8464** (3.37x10^{-6})	**0.0144** (0.0423)	**0.2089** (9.62x10^{-6})	0.0169 (0.572)	0.0081 (0.558)	0 (0.772)
		W	**0.7921** (1.35x10^{-16})	**0.9409** (1.89x10^{-17})	**0.1225** (0.0298)	0.0729 (0.321)	**0.2500** (2.69x10^{-5})	0.1125 (0.328)	0.0009 (0.715)
	LGC12.2	C			**0.1296** (4.38x10^{-7})	**0.3721** (4.79x10^{-7})	**0.0841** (6.61x10^{-6})	0.0049 (0.776)	**0.25** (1.05x10^{-17})
		W			**0.1936** (9.06x10^{-3})	0.0676 (0.338)	**0.3969** (4.75x10^{-6})	0.1296 (0.272)	0.0121 (0.208)
	LGC12.3	C					**0.4096** (9.59x10^{-8})	0.0676 (0.280)	**0.3364** (2.68x10^{-13})
		W					**0.3481** (5.42x10^{-5})	**0.0169** (0.0117)	**0.0484** (0.0494)
	LGC12.4	C							**0.04** (0.0332)
		W							0.0169 (0.215)
CZB	LGC12.1	C	0.2209 (0.0503)	**0.7225** (8.99x10^{-5})	*N/A*	*N/A*	0.01 (0.878)	0.1444 (0.522)	0.0324 (0.340)
		W	**0.4624** (1.54x10^{-14})	**0.8281** (4.30x10^{-30})	*N/A*	*N/A*	0.0001 (0.558)	0.0196 (0.180)	0.0004 (0.904)
	LGC12.2	C			*N/A*	*N/A*	0.0081 (0.671)	0.0625 (0.913)	**0.1125** (6.89x10^{-5})
		W			*N/A*	*N/A*	0.0004 (0.340)	0.0169 (0.384)	**0.0676** (7.65x10^{-5})
	LGC12.4	C							0.0361 (0.190)
		W							0.0036 (0.629)

CZD	LGC12.1	C	0.0625 (0.088)	**0.2916** (0.00105)	*N/A*	*N/A*	0.0009 (0.178)	0.0784 (0.522)	0.0361 (0.175)
		W	**0.0441** (1.04×10^{-10})	**0.1156** (1.84×10^{-16})	*N/A*	*N/A*	0.0881 (0.482)	0.0036 (0.432)	0.016 (0.253)
	LGC12.2	C			*N/A*	*N/A*	0.0049 (0.853)	0.0784 (0.435)	**0.1156** (4.45×10^{-10})
		W			*N/A*	*N/A*	**0.0016** (0.034)	0.0529 (0.0788)	**0.0441** (0.00879)
	LGC12.4	C							0.0225 (0.341)
		W							0.0016 (0.478)

environment for the three inversions, while left-hand cline centres were shifted leftwards across the environmental transition into Wave habitat. The zone of Crab frequency spanned left from the centre of the Crab habitat across the left-hand environmental transition for a short distance into Wave. Inversion clines were less consistent within CZD, but LGC12.4 gave the same displacement as CZB clines, with centres shifted left (left centre in Wave habitat and right centre in Crab habitat) and Crab frequency close to the left environmental transition. The left-hand Wave habitat is also the less exposed in CZD. LGC12.1 and LGC12.2 clines were not clearly discernible but did not appear to be displaced left as above. If anything, one frequency predominated across the transect from the left-land Wave habitat across the Crab habitat, and right-hand clines were close to the environmental transition.

Inversion arrangement frequency differences between ecotypes were compared to the distributions of allele frequency differences (AFD) of collinear SNPs to help show their behaviour in context to the background genetic differentiation. The frequency difference of inversions was variable but often high compared to collinear AFD, although in most cases they did not exceed the 99th percentile (Figure 4). LGC12.4 was the inversion with the greatest AFD in two-thirds of (eight of twelve) scenarios. Low inversion AFD compared to SNP AFD were attributable to LGC12.1 and LGC12.2 where present. Arrangement frequency differences for each inversion were also inconsistent between sexes, sites, and the ecotype comparison pairs within sites. This highlighted the differing behaviour of inversions between the two Wave environments in each site.

Associations between inversions and with sex

While spline model fitting confirmed which inversions showed an overall sex difference across the cline, associations between inversions and sex were calculated within each ecotype to determine whether sex-inversion associations were limited to a specific part of the transect. Inversions that form part of the sex-determining region or that are in LD with it are expected to show significant sex-association. We therefore predicted that, in Crab, LGC12.2 would

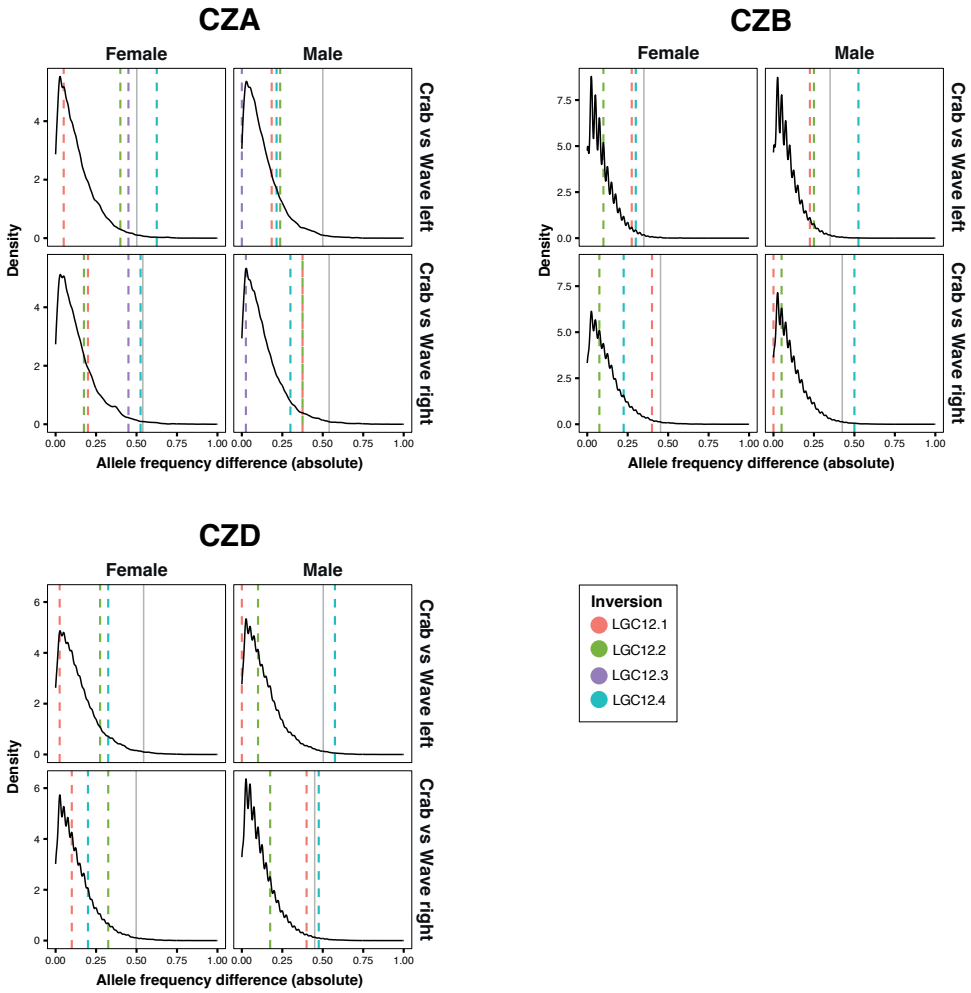

Figure 4. The distribution of absolute allele frequency differences (AFD) of collinear SNPs between Crab and the two Wave habitats for each sex at each site. The solid grey vertical lines indicate the 99th percentile of AFD for each set of comparisons. The dashed, coloured vertical lines show the AFD for each LG12 inversion for each comparison.

show a significant association with sex in all sites and that LGC12.3 and LGC12.4 would show significant associations in CZA, following expectation from heterozygosity and spline analysis results. No strong correlations were expected in Wave although gene flow across the hybrid zone may produce low but significant associations in LGC12.2 (and LGC12.3 or LGC12.4 in CZA), as found in ANG. Indeed, our association analysis revealed the predicted patterns (Table 2). LGC12.2 in all sites and LGC12.3 in CZA were weakly but highly significantly correlated with sex in Crab (r^2 of between 0.11 and 0.37); LGC12.4 was weakly correlated with sex in Crab in CZA only. In Wave, one of the Crab sex-linked inversions was very weakly but significantly associated with sex in each island: LGC12.2 in CZB and CZD, and LGC12.3 in CZA.

Associations between pairs of inversions were also calculated in each ecotype. Inversions that form the sex-determining region are likely to be associated with each other whilst other inversions may not show strong associations. CZA behaved quite differently to CZB and CZD

(Table 2). In CZB and CZD, correlations were generally low and insignificant in both sexes and ecotypes with the exception that LGC12.1 and LGC12.2 were significantly correlated in most cases (Crab females did not show a significant association) (Table 2). This was comparable to the associations found in ANG in Wave (Crab associations could not be calculated). The only other significant association in CZB or CZD was between LGC12.2 and LGC12.4 in Wave females in CZD (Table 2); however, this was only an extremely low, weakly significant correlation (r^2=0.0016, p=0.034) so unlikely to be important. In contrast, many inversion-inversion associations were significant in CZA. This differed between sexes: all bar one pairwise inversion correlations were significant in females while only five of twelve correlations were significant in males (Table 2).

Island-specific patterns of sex-association in the Wave ecotype

As explained above, all linkage groups were examined for heterozygosity differences between the sexes. Linkage group 5 (LG5) alone showed any signal of sex-association in the Wave ecotype in ANG (Hearn *et al*. 2022). Again, if sex-determination systems are shared between islands, sex-association patterns are expected to be replicated across islands. In CZA, LG5 indeed displayed sex-specific heterozygote frequencies (Supplementary Figure 1). Over 90% of the most strongly sex-associated SNPs (the 1% of SNPS with the most negative residuals) in Wave were located on LG5 (Supplementary Figure 1), a highly significant proportion (χ^2=508.88, d.f.=16, p=4.4x10^{-98}). Heterozygosity was once again skewed towards females, as with LG5 at ANG (Hearn *et al*. 2022) and with LG12 in the Crab ecotype. As at ANG, the sex difference on LG5 was not particularly marked, and smaller than the LG12 associations. Residuals reached a maximum of -0.54 at CZA (Figure 2b) and -0.65 at ANG. Strikingly, this pattern was not replicated at CZB or CZD. Not only did LG5 not show any excess of sex differences at these sites, but no sex differences were clearly visible by eye on any other linkage group in Wave (Supplementary Figure 1). Inspection of the most strongly sex-associated SNPs (top 1%) revealed that LG12 was the linkage group that held the greatest proportion on both islands (approximately 20% in CZB and approximately 28% in CZD); LG17 additionally held around 15% of top 1% SNPs in CZB (Supplementary Figure 1). Although not much higher than other linkage groups (which all held up to 10% each of the top 1% SNPs), this was significant at both CZB (χ^2=107.81, d.f.=16, p=1.2x10^{-15}) and CZD (χ^2=119.14, d.f.=16, p=8.0x10^{-18}). Of the approximately 15% of top 1% SNPs in CZB that were located on LG17, 89% (196 of 220) were located within the LGC17.1 inversion (Faria *et al*. 2019). The lack of replication of genotype-sex associations throughout the genome across all four sites in Wave is similar in some manners to the variation in LG12 sex association across sites in Crab, in that sharing across islands is limited for both ecotypes.

The position of high-residual (sex-associated) SNPs was checked across LG5. In both ANG and CZA, sex-associated SNPs were spread across the majority of the linkage group (7.6cM to 45.7cM) with only a small distance at each end lacking these outliers (Figure 2b). Therefore, PCA was carried out on the whole of LG5 for ANG and CZA. Both sites produced very similar PCAs (Figure 5a). PC1 separated a small number of snails (4-5) away from the rest of the individuals in each site. The focal separation of individuals into groups was driven by PC2. Two clear groups without intermediates were present. One smaller group consisted of females only (aside from one male in ANG that could have been mis-sexed during dissection), which were mostly Wave and hybrid zone snails, but also included a couple of Crab snails in CZA. The second, larger, group contained all males and the remaining females.

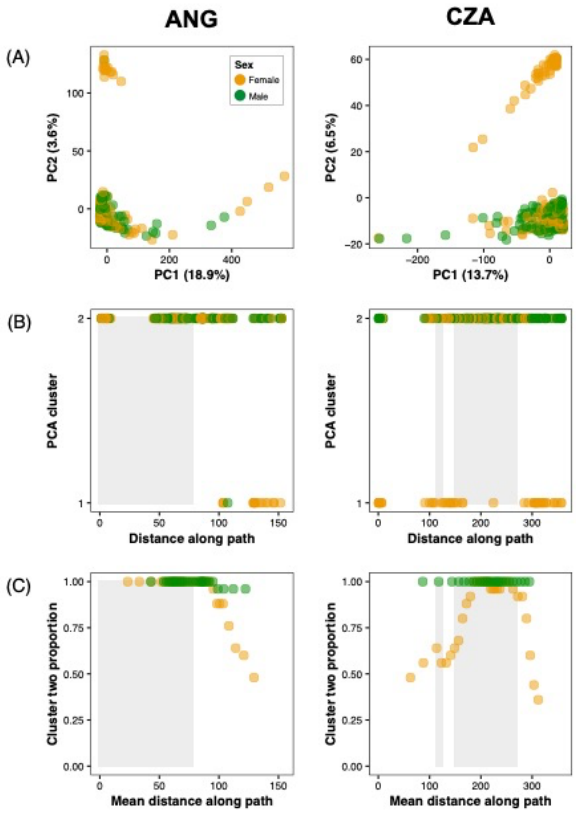

Figure 5. A) PC1 versus PC2 from PCAs (scaled and centred) of LG5 in ANG and CZA. **B)** The distribution along the transects of individuals from the two LG5 PC2 clusters in each site. **C)** The frequency in overlapping, sliding windows across the transects of the more common LG5 PC2 cluster, separately for males and females, in each site. In **A)** and **B)**, the grey shaded regions represent areas of Crab habitat, as in Figure 3; the ANG transect crosses one transition from Crab to Wave, whereas the CZA transect crosses two, from Wave to Crab to Wave.

The distribution of individuals in the two PCA groups and the frequency of putative arrangements was examined along the transect to test for clinal variation. It would also reveal whether the few Crab snails in the small female group were located by the hybrid zone and possibly a result of the boundaries used for ecotype classification for the analysis. Individuals in the small group were distributed relatively evenly throughout the Wave areas of the transect in both sites (Figure 5b). Examining the proportion of individuals in each of the two groups across the transect for each sex revealed a consistent trend in both sites: the small group remained at a proportion of zero in males across the entire transect (almost zero in ANG due to the single male in the small group) and in females the proportion rose from zero in Crab to between 0.5 and 0.8 in the three Wave environments (Figure 5c). In CZA, a small effect of the Crab-like gully on frequency is also visible.

3.5 Discussion

Our study finds evidence that the female-heterogametic sex determination system uncovered in the ANG population of *Littorina saxatilis* is also present in three nearby populations, and is predominant in the Crab ecotype but may also have some influence in the Wave ecotype. The same inversion, LGC12.2 on linkage group 12, forms the sex-determining region in the four sites. However, the association between LGC12.2 and sex in Crab is incomplete; the strength of association is much weaker than in ANG and is variable between the CZ sites. The other three LG12 inversions also vary in polymorphism in addition to their association with sex and ecotype among the CZ sites. Possible associations between other genomic regions and sex in the Wave ecotype additionally vary among CZ sites. We discuss potential explanations for these unique patterns of variation in sex-determination across the populations- including multigenic SD and environment-based sex-specific selection- and implications for the wider *L. saxatilis* distribution.

Sex-determination and sex-linked inversions in the Crab ecotype

Sex differences in heterozygosity confirmed that a ZW (female-heterogametic) sex-determination system was present in all three CZ sites. Sex-associated SNPs were also clustered on linkage group 12, the location of an SD locus in ANG, thus suggesting that the same sex-determination system is shared across the four populations. However, the specific distribution of sex-associated SNPs along LG12 indicated variation in the sex-determining region (SDR): in CZB and CZD the SDR ended at 43.8cM whereas it reached 48.7cM in ANG and CZA (at all sites the SDR began at 33cM). Concurrently, the LGC12.2 inversion (33-43.8cM) was present and sex-associated in all CZ sites whereas LGC12.3 (43.8-48.7cM) was present and sex-associated in only CZA. The SDR in ANG and CZA is covered by the two adjacent inversions but is restricted to the location of LGC12.2 in CZB and CZD. This may suggest that LGC12.3 is younger than LGC12.2 and first evolved in ANG or CZA and has not yet spread to the other sites. Another possibility may be that population-based differences in sexual antagonism (Connallon and Clark 2014; Connallon 2015) of a trait whose locus lies in the region of LGC12.3 has driven expansion of the SDR in CZA and ANG only, through the evolution of LGC12.3.

Furthermore, sites varied in the strength of sex association of SNPs in the SDR. Associations at all CZ sites were much weaker than at ANG. This was the case despite a lack of recombination between LGC12.2 (and LGC12.3 in CZA) inversion arrangements, as indicated by an absence of intermediates between the three distinct groups on PCAs. The weaker patterns of association were therefore not a result of recombination eroding the sex association of SNPs. Variation in the strength of sex association was also evident in the frequency of inversion arrangements in each sex: arrangements showed a smaller sex difference in frequency in the CZ sites than in ANG. This could overall suggest that the SDR is younger in sites where the sex difference is smaller as the sexes have been diverging for a smaller amount of time so have accumulated fewer differences. As discussed later, differing levels of gene flow between ecotypes can also affect the patterns of sex association that are visible. However, it is likely the SD locus is not completely associated with LGC12.2, i.e., both the male and female alleles at the SD locus are present on at least one of the two LGC12.2 arrangements, and these associations differ between sites. Other SD loci may also be contributing to sex determination to different extents across sites, decreasing the effect of the SD locus in LGC12.2. Both effects decouple patterns of inversion frequency between the sexes from patterns of allele frequencies at the SD locus; thus it is not possible to understand behaviour of the SD locus solely based on the inversions. The use of capture data gave only

limited coverage so it is unlikely the SD locus itself is part of the dataset, precluding the ability to distinguish between incomplete LD of the SD locus with LGC12.2 and the presence of other SD loci.

Divergently selected inversions between ecotypes

Analysis of LD clusters using PCA confirmed that the LG12 inversions first identified in ANG are present across the CZ sites and splines established patterns of frequency change between ecotypes. Inversion frequency differences between ecotypes were often higher than most collinear SNP allele frequency differences (AFD), supporting stronger divergent selection between ecotypes on the inversions than the genetic background. However, high AFD might be due to drift/barrier effects and low AFD might be maintained by balancing selection, so the AFDs can support but not give evidence of selection on inversions. In ANG, LGC12.1 was the only inversion of the four to show no sex differences. Across the CZ sites, LGC12.1 behaved in a similar manner to ANG: no sex differences were detectable and clines in frequency were present between the ecotypes. It would therefore appear that this inversion is divergently selected between ecotypes and is important for ecotypic differentiation, as has frequently been found in other species (e.g. McAllister *et al*. 2008; Joron *et al.* 2011; Wang *et al.* 2013; Lee *et al.* 2017). This is supported by the clines generally following a similar form across sites, including shared frequencies within ecotypes, which further implies that arrangements are under similar selection at all sites. CZD, however, is an exception to this. The lack of distinct cline at the left-hand transition would suggest that selection on this inversion is relaxed or in a different direction in this Wave habitat as the arrangement at a high frequency in Crab also persists at a high frequency into Wave here. This side of the shore is more sheltered than the other, reducing wave action and making it more Crab-like; it is possible that this is driving the more Crab-like inversion frequency through the effect of an adaptive locus within it. However, other sites also differ in exposure between the left and right Wave habitats and do not show this distinct inversion frequency trend. Westram *et al*. (2021) reported that allele frequencies and inversion-cline end frequencies were generally similar across zones, although differences in outlier SNP frequencies between left and right hybrid zones appeared more prominent in CZD than the other sites (Figure 4 in Westram *et al*. (2021).

The differential presence and absence of LGC12.3 across sites placed us in a position to disentangle the role of sex- and ecotype-specific selection on LGC12.4. In ANG, LGC12.4 showed some sex difference in frequency in Crab, although it was unclear whether LGC12.4 was part of the SDR or merely in some LD with it. Spline fitting confirmed that LGC12.4 also displayed different frequencies in males and females in CZA where LGC12.3 is present, whereas no sex difference was present in CZB or CZD where LGC12.4 was absent. This therefore implies that the sex difference is produced by the proximity of the two inversions and so a build-up of LD between them, rather than a specific role of LGC12.4 in the SDR. The cline in frequency between ecotypes remains comparable across the four sites regardless of the sex effect; divergent selection is acting similarly at all sites and is independent to the effect of LD with the SDR. Of note, LGC12.4 shows a clear cline in CZD, which is similar to the other sites, despite LGC12.1 not showing a cline as previously mentioned. This would suggest that the lack of cline for LGC12.1 is specific to a locus in this region rather than a more general selective effect across LG12: inversions influence different phenotypes according to the loci within them (Koch *et al*. 2022), and so respond to different components of habitat-based selection.

The sex-linked inversions (LGC12.2 and LGC12.3) show ecotype differences in frequency as well as sex differences. Once again this is replicated across sites, demonstrating a role of divergent selection in combination with sex-specific selection in shaping the clines of these inversions. The noticeable effect on inversion frequencies in the sexes of the sheltered gully in CZA - offering Crab-like habitat over a very small distance- highlights the divergent selection on LG12 between ecotypes. As with LGC12.1, inversion frequencies in Crab persist into the left-hand Wave environment in CZD, possibly in relation to adaptive selection based on wave exposure differences (see above). Local adaptation has previously been suggested to be sex-specific in other species; for example, an association between abiotic environmental variables and differences in sexually dimorphic traits was demonstrated in the plant *Rumex hastatalus* (Puixeu *et al*. 2019). Further, in a pair of *R. hastatulus* populations that are joined by a hybrid zone and differ by a fusion that produced a neo-X chromosome in one population, SNPs with the steepest clines all map to the neo-X (Beaudry *et al.* 2022); the sex chromosome is most strongly divergently selected genomic region. The theoretical relationship between local adaptation and sexual antagonism (leading to sexual dimorphism) has also been investigated (Connallon and Clark 2014; Connallon 2015), where it was concluded that the intensity of sexual antagonism can vary across species' distributions and that in dioecious species, sexual antagonism is an "inescapable by-product of adaptation". LGC12.3 follows the same clinal pattern in CZA as ANG, again suggesting concurrent selection acting in the two populations. The strong separation of the ecotypes by PC1 in the LGC12.3 region in CZD despite the lack of inversion is surprising, especially since no such pattern in visible in CZB. Due to little overlap between SNP sets between the sites, it was not possible to check whether the same SNPs were important in separating the ecotypes in CZD as in CZA.

Clines of LGC12.2, however, vary greatly in shape between sites, and sex differences persist across some of the transitions into Wave habitat. Migration or gene flow of individuals from Crab into Wave can make Wave frequencies more Crab-like; it is possible that this is playing a role here, rather than a low level of sex-specific selection in Wave, as the male and female differences tend to decrease at distant ends of the Wave habitats away from the hybrid zones. However, as the SD locus does not appear to be completely associated with LGC12.2 and this varies between sites, the inversion can act partially independently from the SD locus in response to selection and gene flow. Therefore, variation in LGC12.2 clines between sites may not be reflective of the behaviour of the SD locus itself.

Among sites, the location on the transect of cline centres and therefore regions of 'Crab' arrangement frequencies of inversions varied in relation to habitat (Figure 3). Each inversion within a site showed the same cline centres (approximately) and Crab frequency regions, indicating that selection pressures are aligned between inversions and likely relate to the same change in habitat. In ANG, clines of inversions on LG12 and other linkage groups are all shifted to the right of the main environmental transition such that cline centres are all in the Wave habitat. One might therefore expect cline centres to all be shifted into Wave at both left and right hybrid zones in the CZ sites; this would produce a wider region of Crab frequency than the distance between the main environmental transition at each hybrid zone, as is seen at these sites for many loci and inversions (Westram *et al*. 2021). However, this is not the case. At each site, both cline centres are shifted in the same direction (both left or both right) so the Crab zone of frequency is narrower than the Crab habitat and shifted over close to one of the transitions. This may be due to a specific aspect of the environment driving the change in inversion frequencies between ecotypes that does not change in the same location on the

shore as the more prominent aspects of the shift in habitat. It also suggests that the pair of transitions in each site may be quite different from each other despite their proximity. This is demonstrated by the cline differences between left and right transitions for LGC12.1 and LGC12.2 in CZD; SNP cline centres were also shifted further into Wave in the left than right transition in Westram *et al*. (2021). The transition in habitat at the right side of CZB is more gradual than the other hybrid zones, and inversions and SNPs both show cline centres much more displaced into Wave for this transition than the left (Westram *et al*. 2021); concurrently, LG12 clines are less steep on the right than left in CZB, especially for LGC12.4. Asymmetric gene flow between ecotypes and across each hybrid zone may contribute to the cline shifts. The predominantly different behaviour of LG12 compared to other genetic regions (Westram *et al*. 2021) supports the suggestion that this pattern is produced by selection on LG12, rather than density or dispersal effects, which would impact clines of all genomic regions similarly.

Sex differences in the Wave ecotype

In contrast to the sex determination system in the Crab ecotype which was dominated by LG12 across all four sites, although to varying degrees, the LG5 sex linkage detected in Wave in ANG was replicated only in CZA (Supplementary Figure 1). As in other analyses, there is a large difference between these sites and the other two sites. LG12 held the greatest proportion of the top 1% of the most strongly sex-associated SNPs in Wave in both CZB and CZD (Supplementary Figure 1). Since gene flow is operating in all four sites yet the sex-association of LG12 in Wave is present in only two sites, this is suggestive of the LG12 SD locus playing some role in sex determination in Wave as well as Crab in CZB and CZD. However, LG17 also showed a potential peak in top 1% SNPs in CZB (Supplementary Figure 1). The majority of these SNPs on LG17 fell into the LGC17.1 inversion (Faria *et al*. 2019), which shows strong frequency differences between the ecotypes, is enriched with outlier SNPs in ANG (Faria *et al*. 2019; Westram *et al*. 2021) and is associated with adaptive traits (Koch *et al*. 2021). It is not clear at this stage whether an SD locus is present in this region in Wave in CZB or whether there is LD between LGC17.1 and an SD locus elsewhere (probably maintained due to environmental selection of some kind). Further investigation is required to clarify, but the importance of this genomic region in adaptive divergence highlights a possible interplay between this and sex determination and makes for an interesting candidate region for further study.

LG5 behaved similarly in ANG and CZA (Figure 5). Sex-associated SNPs were spread across the majority of LG5, and PCAs of the linkage group produced two main clusters which generally separated Wave females from all other snails (Figure 5a-b). This was an analogous grouping to LGC12.3 in ANG and CZA (but with ecotypes reversed) and the frequencies of putative arrangements also followed a similar clinal pattern albeit less distinct (Figure 5b-c). These arrangements reached roughly 0.5 frequency in Wave females- with no alternative homozygotes present despite the 0.25 frequency expectation, as is expected from a female-dominant SD locus- whilst one arrangement was fixed in males and Crab females. As with the Crab sex-determination system on LG12, this is akin to what is expected for a sex-linked inversion in a ZW system in the Wave ecotype that is not polymorphic in Crab. Whilst this is suggestive of an inversion spanning the majority of LG5 in ANG and CZA, we do not test for this specifically in this study so cannot conclude that an inversion is present. The behaviour of LG5 would match that expected of a young, little-differentiated inversion at a low frequency in the populations, lacking homozygotes of the alternative arrangement due to its role in sex determination; the combination of these characteristics would cause difficulty in detection. The

long span of an inversion across the length of LG5 could also reduce its efficacy in suppressing recombination, limiting differentiation and detectability; however, as diagnostic SNPs are spread across the length of LG5, this seems unlikely in this case. Previous inversion detection in ANG (Faria *et al*. 2019) did not find evidence for an inversion on LG5. However, in that study, inversion detection was carried out without separation of sexes or ecotypes; with the potential inversion only polymorphic in a small subset of individuals (Wave females), it is possible that this obscured any signals of an inversion. Analysis of sex-specific LD patterns and recombination, as carried out on LG12 (Hearn *et al*. 2022), is required to support the presence of an inversion on LG5.

Pairs of closely related species or populations of a species have frequently been shown to differ in their sex-determination systems, and these often involve chromosomal rearrangements. However, examples overwhelmingly involve established, heteromorphic sex chromosomes, their fusions or translocations and their degeneration (e.g. stickleback (Kitano *et al.* 2009); frogs (Miura *et al.* 2012); butterflies (Smith *et al*. 2016); beetles (Bracewell *et al.* 2017)) rather than young, labile SD systems (potentially with multiple different SD loci) and polymorphic inversions.

Differences between populations

It is evident across multiple analyses that, despite some sharing of sex-determination system and inversions, many differences exist between the four populations. None of the CZ sites exhibits as strong or clear sex or ecotype associations as in ANG. Whilst this could be a sign of the age of the sex-determination system or contributions from other SD loci in each site, aspects of the sampling strategy may have contributed to the dilution of some patterns. As mentioned previously, the transect at each CZ site spanned two hybrid zones and Wave habitats rather than the single transition at ANG. The same numbers of individuals were collected at all four sites, leading to approximately 50% more sparse distribution of snails along the transect in the CZ sites than ANG. Windows to calculate inversion frequencies were a fixed number of snails, so each window covered a much greater distance along the shore and therefore a greater variance in habitat and ecotype near the hybrid zones. Fewer windows will be ‘pure’ Crab or Wave, contributing to frequencies in windows being more variable and obscuring clines.

Differences in features of the habitats between sites can also affect the strength of sex-association detected. The width of Crab habitat between the pairs of hybrid zones varied greatly between sites: around 100m in CZA, 70m in CZD and just 20m in CZB (Figure 1; Figure 3 grey shading). Unusually for the area, the Crab habitat in ANG is much longer even than in CZA. It is these sites, ANG and CZA, with the longest regions of Crab habitat that show the strongest associations of inversions with sex in Crab. Migration and gene flow across the hybrid zone will affect a greater proportion of Crab habitat in some sites than others, even without differences in rates between sites. A smaller region of ‘pure Crab’ individuals will once more affect the frequencies calculated in windows across the transect and affect analyses conducted on the two ecotype groups. Similarly, where cline centres are displaced into Wave, especially in a narrow Wave region, this may leave little area of ‘pure’ Wave genotypes on the transect.

ANG and CZA show several likenesses that separate them from CZB and CZD; namely, the presence and behaviour of LGC12.3 (and subsequent sex-association of LGC12.4) and sex-

specific patterns on LG5. It is not surprising that CZB and CZD are similar to one another as the distance between the two sites is very small (0.4km); however, ANG and CZA have the greatest pairwise distance between them (Figure 1) yet are the most similar to one another. It is clear that the relationship between sites is not solely being driven by distance and gene flow. The level of similarity between pairs of sites also differs from the sharing of SNP cline outliers including and excluding inversion regions (Westram *et al*. 2021), where ANG and CZA have one of the lowest levels of sharing.

It is unclear at this stage what the main driver is for patterns of similarity between sites. As above, distance between sites is unlikely to be the only aspect playing a role. Variance in habitat may be associated with similarities in patterns between sites, i.e. a specific aspect of the environment within 'Crab' and/or 'Wave' habitats. In sockeye salmon, complex relationships of differentiation between sexes, environments, and sites have also been evidenced (Oke *et al.* 2018). Although phenotypic rather than genetic variables were tested (and sex determination is not discussed), patterns are analogous to those in *L. saxatilis*-sexual dimorphism (sex differences) differs between beach and creek breeding (ecotypes) and this further varies between lake systems (sites). They also find that site-variation is associated with variation in an environmental factor. As all four inversions on LG12 are involved in local adaptation between Crab and Wave habitats, as evidenced by the clinal variation in frequency between ecotypes in addition to their association with outlier SNPs and adaptive traits (Koch *et al*. 2021; Westram *et al*. 2021; Koch *et al*. 2022), differences in the habitat can produce selection for different arrangement frequencies in each site. LG12 is also important in adaptation to shore height (Morales *et al*. 2019) so selection is likely to be driving arrangement frequency differences along this environmental axis simultaneously to the Crab-Wave axis. If these axes are not spatially concurrent on the shore at all sites, patterns of inversion frequency changes will become more complex and may differ within ecotypes across sites. Moore *et al.* (2022) show that polygenic sex determination in a Lake Malawi cichlid species produces modular sexual polymorphism on a higher order than simple sexual dimorphism usually associated with single-locus sex determination. In this manner, multigenic sex determination can allow additional axes of differentiation; it is possible that a similar process is occurring in *L. saxatilis*, where varying importance of different SD loci and sex-linked inversions across ecotypes and environments facilitates complex differentiation across nonconcurrent environmental and selective axes. The age of an inversion or sex-determination (SD) system and their route of spread between populations can also affect the strength of relationships detected. A young inversion or SD system that has newly appeared in a population will not show frequency differences as strong as in a population where it's well-established if it has not yet had time to spread throughout the population (Furman *et al*. 2020). The patterns observed between ANG and the CZ sites would suggest that the SD system and inversions appeared first in ANG, followed by CZA and then CZB and CZD under this scenario. This is possible with independent origins in each population or their spread through migration (if the levels of migration between sites match the order of appearance). It is likely that a combination of the above factors is working in concert to shape the patterns between sites observed on LG12. The incomplete associations among inversions and between inversions and sex are a reminder that each inversion and the SD system may have independent, unique paths of spread. Moreover, the association patterns between groups may be an indicator that other SD loci are contributing to sex determination to differing extents across ecotypes and sites, as supported by variation in sex-association across multiple linkage groups

(Supplementary Figure 1). This limits the utility of hypotheses of order of origin or route of spread between sites.

There remains much that we do not understand of the sex-determination system(s) in these populations of *L. saxatilis*. Patterns of frequency and association between inversion, sex and ecotype are clearly very complex and vary within and between populations. Exploratory analyses confirm that the sex-determining system in the Crab ecotype is not restricted to ANG, but multiple differences between populations highlight a need for further study to understand sex-determination in this species. It is likely that both which locus (or loci) is involved in sex determination and the amount of association between the SD locus (or loci) and the inversions in each site and ecotype are contributing to the variation displayed. A multigenic sex determination system where association with adaptive inversions differs across environments, or differing sex determination systems across environments associated with adaptive inversions, or a combination of the two, may be occurring. It is clear that sex-determination must be considered alongside adaptive divergence and the evolution of inversions to understand the many roles of LG12 in *L. saxatilis* but also how those processes can interact.

As previously mentioned, isolated aspects of the *L. saxatilis* system support multiple broad evolutionary patterns that are seen across a range of species: clinal variation in inversions associated with adaptive divergence; inversions involved in sex chromosomes; and sex chromosome differences between populations and their role in reproductive isolation. However, the specific features evidenced here and their combination in one system provides a truly unique example. Studies on differing sex determination systems across populations/species involve fusions, translocations, or degeneration of heteromorphic sex chromosomes and one SD locus (e.g. Miura *et al.* 2012; Bracewell *et al.* 2017; Katsumi *et al.* 2022); neither are links to environmental differences or the role of ecology in sex-specific selection examined. Where sexual dimorphism across environments has been the subject of studies, genetics has not been included (e.g. Oke *et al.* 2018) or is in a system where established sex chromosomes have undergone a fusion (e.g. Puixeu *et al*. 2019; Beaudry *et al.* 2022). At this point, no other system comparable to *L. saxatilis* has been identified; the variation in a young labile SD system that is tightly linked to adaptive inversions across environments within and between populations is exactly what has been called for and will be valuable for future work (Furman *et al.* 2020).

Multiple systems can be segregating in the same population or species, and they can be associated with inversions, but this relationship can be unstable and evolve. Multigenic (smaller numbers of loci, as opposed to polygenic with many) SD is also frequently considered to be an unstable state between ancestral and derived SD systems (Feller *et al*. 2021) and few examples have been found in wild populations (Yusa and Kumagai 2018). Multigenic SD has been described in apple snails (Yusa and Kumagai 2018) and a Lake Malawi cichlid species (Moore *et al.* 2022), but only single populations were studied. New modelling shows theoretically that if expression of genes within SD regulatory pathways is affected by the environment, divergence of genetic SD mechanisms within species can occur and may result in the spatial variation in the occurrence of multiple coexisting SD mechanisms (Schenkel *et al.* 2023). Multigenic or multiple SD systems- both potentially occurring in *L. saxatilis*- and their driver are poorly understood and largely unknown. Better understanding of the *L. saxatilis* system and its utilisation to test theoretical ideas of drivers and mechanisms of early sex

chromosome evolution and the role of ecology may also help shed light on how these complex SD systems evolve.

The underlying causes of a number of results remain unclear due to the nature of the data used in this study and in Hearn *et al*. (2022); future work in this area should incorporate both more specific sampling and analyses (within populations) and more wide-ranging studies (including many more populations across the species range). A combination of understanding the specific causes of patterns within certain populations as well as drivers across the species more broadly will together help to understand the processes occurring. Use of whole-genome sequencing (WGS) data gives great potential for insight. Single loci (e.g. the SD locus) are easily missed in data such as that used in this study, hence the need to rely on inversions (where the long-range LD allows detection of the SDR). WGS data, in contrast, should allow separate identification of inversion and SD system patterns. The incomplete associations between inversions and sex in these *L. saxatilis* populations make for a powerful system in which to separate and understand their different effects.

3.6 References

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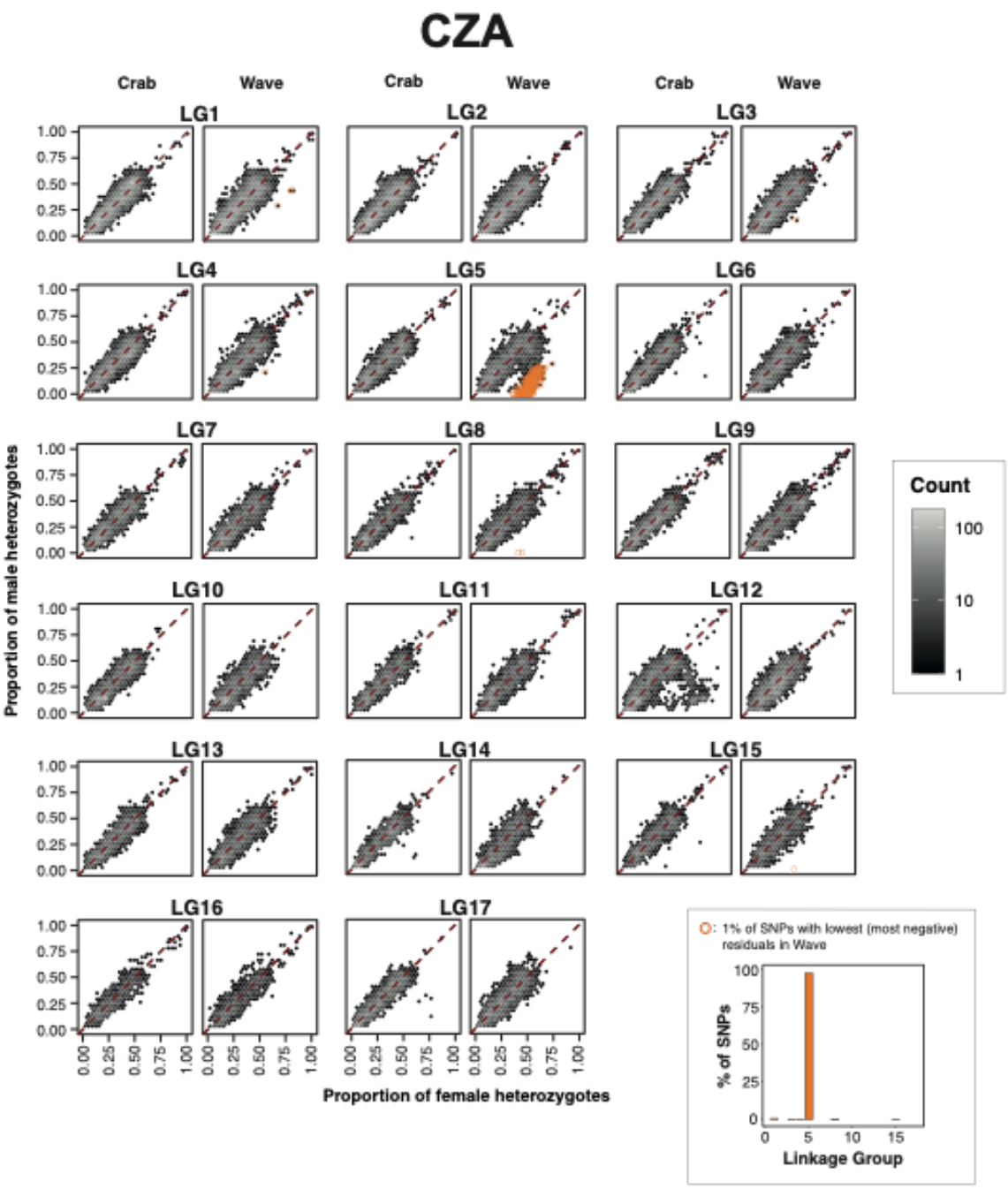
3.7 Supplementary Results

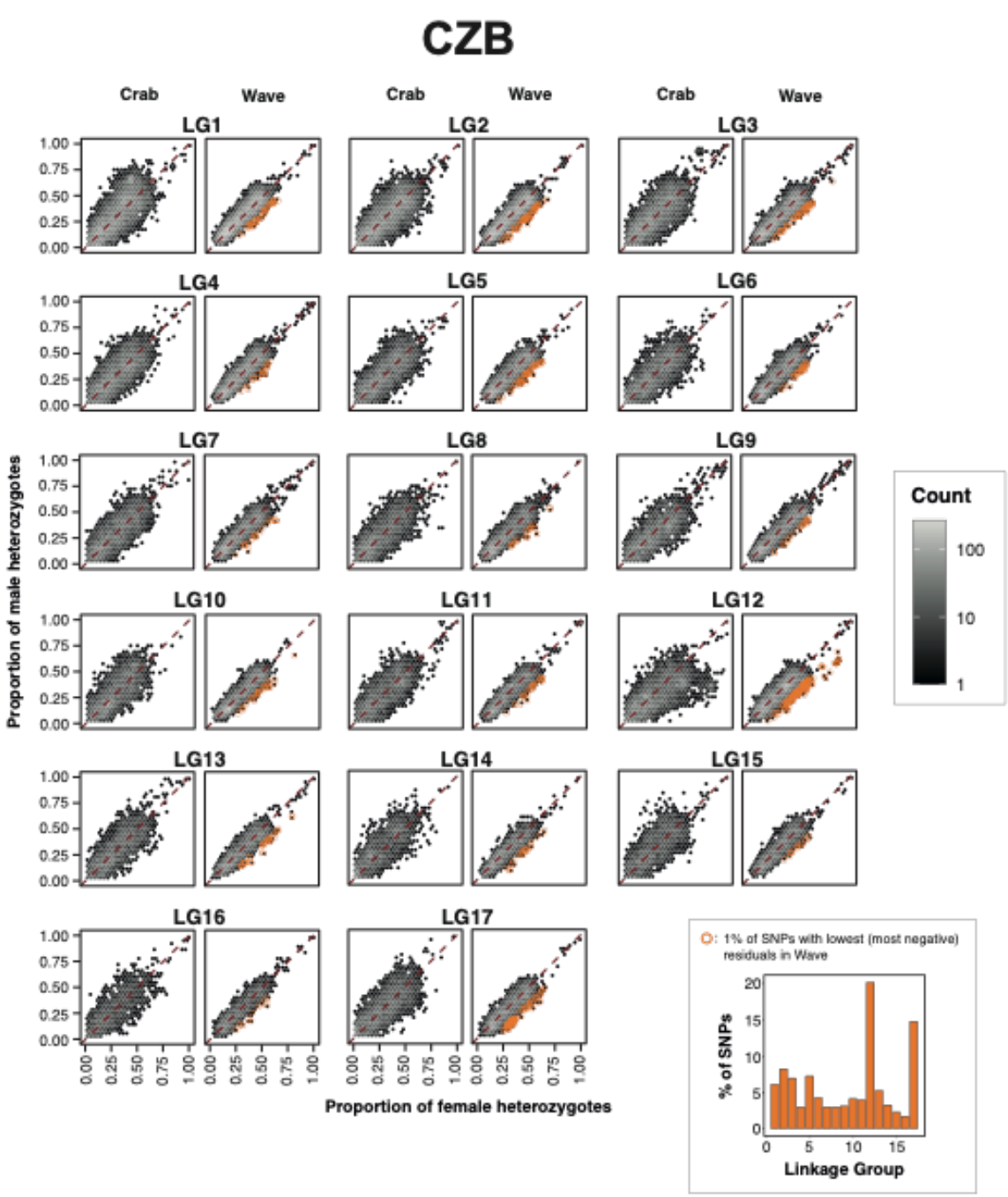
Putative inversions on the Crab sex chromosome

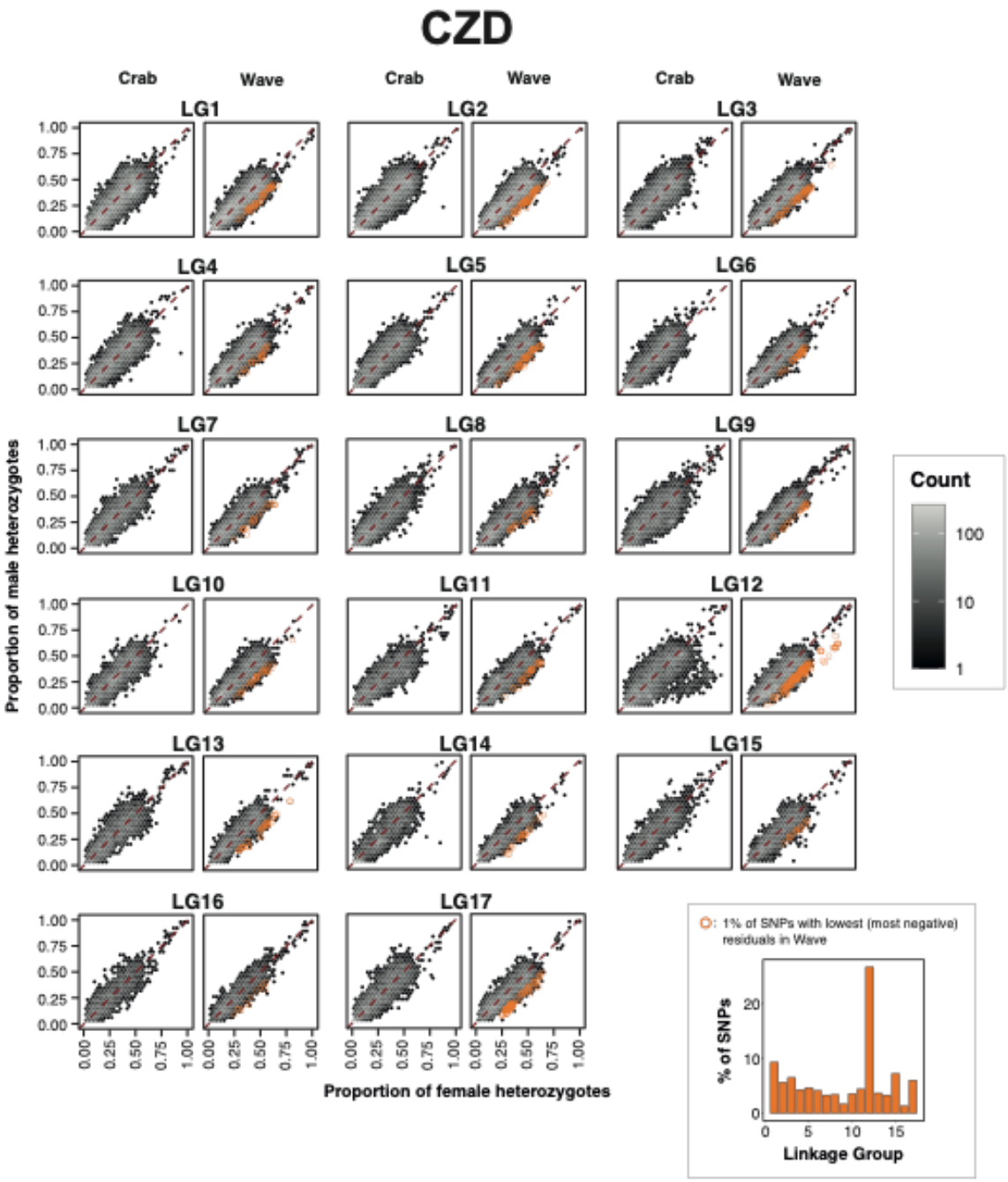
Inversion detection was initiated using the R package LDNA to investigate patterns of linkage disequilibrium (LD) between SNPs on LG12. The parameters $|E|_{min}$ and φ were manipulated, as in Hearn *et al*. (2022) to produce lists of outlier clusters of SNPs under high LD. In all sites, many clusters were detected across the range of parameter combinations. However, numerous clusters appeared only sporadically at specific parameter combinations so were not retained. Of the few that appeared repeatedly at many combinations of parameters, clusters often did not pass the criteria for a cluster that could be indicative of an inversion; the criteria were the same used as in Hearn *et al*. (2022). In this case, it was commonly as all SNPs in a cluster were from a single map position or sparsely scattered across the linkage group (rather than clustered in one region), or as multiple clusters at lower parameter combinations were part of single larger clusters at other combinations.

At CZB and CZD, three main clusters of interest appeared recurrently and were retained for downstream analysis. They covered the same three regions of LG12 in both sites, which also matched the locations of the LGC12.1, LGC12.2 and LGC12.4 inversions in ANG. In CZA, four main clusters were identified. One matched the LGC12.4 region as in the other three sites, and another was a similar match for the LGC12.1 region but extended a further 2cM than in the other sites. The other two clusters retained in CZA appeared alternately at different parameter combinations, but one spanned a subset of the region that the larger covered. One cluster matched the location of LGC12.2 (as in CZB and CZD) but often appeared as the larger cluster of 33-48cM- both LGC12.2 and LGC12.3 regions together. To test which cluster to retain, PCA was carried out on both the smaller and larger region, as well as the LGC12.3 region as an additional test. The PCAs of both the individual ANG inversion regions produced three distinct groups along PC1 whereas a PCA of the combined region gave six groups. This was an indicator of two separate inversions, but with some LD between them which caused them to be detected as a single cluster under some parameter combinations. Therefore, for CZA, the LGC12.2 and LGC12.3 regions were considered separate clusters- giving four cluster regions for the subsequent analyses.

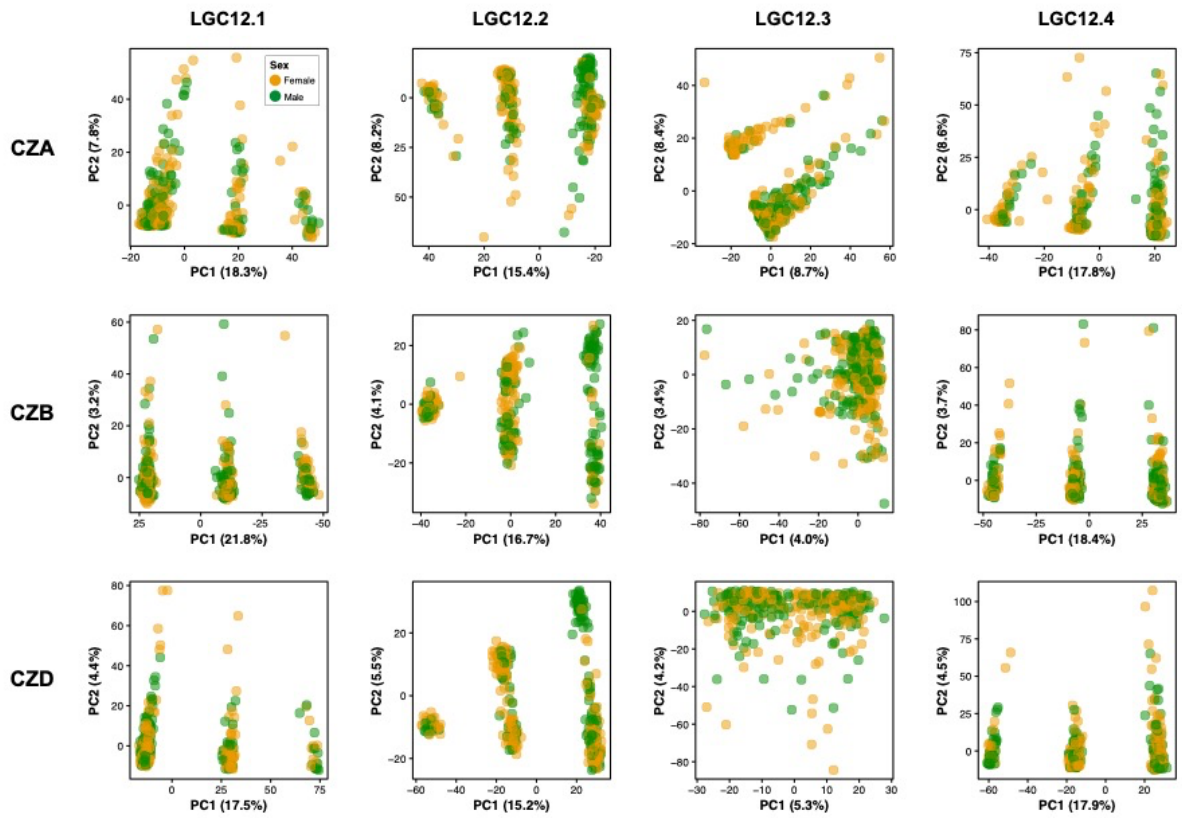
3.8 Supplementary Figures




CZB
Crab
Wave
LG1
LG2
LG3
LG4
LG5
LG6
LG7
LG8
LG9
LG10
LG11
LG12
LG13
LG14
LG15
LG16
LG17
Proportion of male heterozygotes
Proportion of female heterozygotes
Count
100
10
1
○: 1% of SNPs with lowest (most negative) residuals in Wave
% of SNPs
Linkage Group



Supplementary Figure 1. The proportions of each sex in the two ecotypes that are heterozygous at SNPs on the 17 linkage groups in CZA, CZB and CZD. Hexagons with a greater density of SNPs are shaded lighter grey. The 1:1 line represents the neutral expectation of no sex difference, and SNPs with greater sex differences are further from this line. Orange circles represent the 1% of SNPs with the most negative residuals (furthest below the 1:1 line) in Wave; their distribution across linkage groups is shown in the inset.



Supplementary Figure 2. PC1 versus PC2 from PCAs (scaled and centred) of SNPs in the four LG12 inversion regions in CZA, CZB and CZD.

3.9 Supplementary Tables

Supplementary Table 1. Distances along each one-dimensional transect used to classify snails into ecotype groups. Snails within 10m of the main environmental transitions (Westram *et al*. 2021) were classified as hybrids; the remaining snails were classified by habitat (rocky cliff: Wave; boulder field: Crab). All distances are in metres. Numbers in brackets in the hybrid columns indicate the environmental transitions.

Island	Wave left	Hybrid left	Crab	Hybrid right	Wave right
CZA	<139	139-159 (149)	159-259	259-279 (269)	>279
CZB	<80	80-100 (90)	100-121	121-141 (131)	>141
CZD	<81	81-101 (91)	101-172	172-192 (182)	>192

Supplementary Table 2. The number of snails of each ecotype and sex classification on each island, classified according to position along transect (Table 1). Totals are also given for sex, ecotype, and overall.

Island		Crab	Hybrid	Wave	Total
CZA	Female	93	20	78	191
	Male	85	27	60	172
	Total	178	47	138	363
CZB	Female	43	33	133	209
	Male	34	30	108	172
	Total	77	63	241	381
CZD	Female	62	30	108	200
	Male	57	24	88	169
	Total	119	54	196	369

CHAPTER FOUR
-
Habitat choice, divergent selection, and the role of inversions in local adaptation in *Littorina saxatilis* ecotypes

4.1 Abstract

While it is accepted that divergent local adaptation can occur in the face of gene flow, understanding of the process remains incomplete. When multiple mechanisms and drivers occur in concert and create linkage disequilibrium between contributing factors, the extent of reproductive isolation and barriers to gene flow are greater. When dispersal (and so gene flow) is non-random and results in individuals spending a greater proportion of time in habitats where they have higher fitness, i.e., individuals exhibit habitat choice towards environments to which they are locally adapted, this can drive assortative mating, promote adaptive divergence, and drive reproductive isolation. Divergent populations often differ by chromosomal inversions and they are thought to increase the barrier to gene flow through linkage of reproductively isolating traits. Such traits frequently show sex-specific effects on fitness (sexual antagonism) and are regularly located on sex chromosomes or linked to sex-determining loci, often within inversions. Therefore, sex determination mechanisms and sex-specific selection are thought to contribute to adaptive divergence and the same inversions may play a role in both processes concurrently. To investigate these processes, reciprocal-transplant mark-recapture experiments were carried out in two Swedish populations of the intertidal snail, *Littorina saxatilis.* Differential survival, movement, and changes in phenotypes and inversion genotypes were examined across ecotypes and habitats. The results show evidence of divergent selection through reduction in survival of ecotypes away from their home environments and crossing reaction norms, a hallmark of local adaptation. Further, ecotypes showed differing non-random dispersal between habitats through direction and distance of movement, indicating habitat choice. Arrangement frequency of sex-linked inversions differed according to sex, ecotype, and habitat, highlighting sex- and habitat-specific divergent selection acting upon them and their importance in both local adaptation and sex determination. Altogether, I provide evidence for multiple components of reproductive isolation between ecotypes and a role for sex-linked inversions.

4.2 Introduction

Speciation in the face of gene flow is now widely accepted to occur, yet the process is not fully understood (Foote 2018). Natural selection on populations inhabiting different environments can lead to local adaptation and, if the strength of selection is great enough, the build-up of isolation between the populations (Coyne and Orr 2004). Generally, divergent selection must be stronger than gene flow between populations to facilitate divergence (Rice and Hostert 1993; Smadja and Butlin 2011). Selection against locally adapted individuals when in a contrasting habitat becomes visible through a reduction in fitness, i.e. survival and reproduction (Nosil *et al*. 2005). Therefore, factors that reduce the amount of time that individuals of a locally adapted population spend away from their 'home' environment are favoured.

Despite dispersal, as a component of gene flow, often being considered an opponent to local adaptation, non-random dispersal and gene flow can play a key role in the differentiation of populations (Edelaar *et al*. 2008). Non-random dispersal, specifically where movement behaviour results in individuals spending more time in certain environments than would be expected under random dispersal, is usually referred to as habitat choice (Futuyma and Moreno 1988). It should be noted that this is a poor term, however, as no active comparison and choice is needed. Habitat choice can contribute to the divergence and speciation process through a number of paths in which it affects non-random mating and increases reproductive isolation (Webster *et al*. 2012). When individuals move into, and subsequently breed in, habitats in which they have greater fitness, they are likely to mate with similar conspecifics to themselves (Kopp *et al*. 2018). This is effective as a barrier to gene flow between groups that are locally adapted to disparate environments (Maynard Smith 1966; Rice 1984a; Camacho *et al*. 2020). In this way, habitat choice drives assortative mating and reinforces reproductive isolation.

Multiple other factors aside from habitat choice are involved in, and usually work in concert to promote, reproductive isolation between divergently selected populations. Barriers to gene flow in the genome appear and accumulate around the loci for such locally adaptive and reproductively isolating traits (Butlin 2010). Genetic architecture that increases the association between these loci increases the genomic extent of the barrier to gene flow (Ravinet *et al*. 2017). Chromosomal inversions that differ between closely related species have been widely documented and their role in local adaptation and divergence frequently discussed (Joron *et al*. 2011; Wang *et al*. 2013; Jackson *et al*. 2016; Lee *et al*. 2017). Suppression of recombination between locally adaptive loci by inversions maintains different combinations of alleles in each arrangement beneficial for the divergent environments. Hybrid offspring with recombinant genotypes are therefore maladapted to both environments and suffer reduced fitness (Kirkpatrick and Barton 2006; Kirkpatrick 2010). In this manner, inversions increase the barrier to gene flow between the populations, especially if reproductively isolating loci in addition to locally adaptive loci are captured in the inversion (Fuller *et al*. 2018).

Traits involved in reproductive isolation are often sexually antagonistic- they offer contradictory fitness effects in males and females. For example, mate choice cues can be sexually antagonistic and can lead to assortative mating between diverging populations that differ in those traits (Qvarnström and Bailey 2009; Rabosky 2016). Loci for sexually antagonistic (and reproductively isolating) traits are often located on sex chromosomes (e.g. Kitano *et al*. 2009; Smith *et al*. 2016; Bracewell *et al*. 2017); they are hypothesised to drive the evolution of sex chromosomes themselves but established sex chromosomes are also fertile grounds for the accumulation of such loci (Rice 1984b; Charlesworth *et al*. 2005). The role of sex chromosomes in speciation is well-known, through processes including the large-X and faster-X effects and Haldane's rule (Charlesworth *et al*. 1987; Charlesworth and Charlesworth 2000; Coyne and Orr 2004; Presgraves 2008; Lasne *et al*. 2017). Loss of recombination on the sex chromosomes is key in the above processes, of which inversions are a predominant mechanism (Wright *et al*. 2016). As such, inversions on sex chromosomes are known to be important in divergent selection between populations.

The intertidal snail *Littorina saxatilis* is an ideal system for the study of divergence in the face of gene flow (Johannesson *et al*. 2020). It occupies highly heterogenous habitats with sharp

environmental gradients across small distances. Distinct ecotypes have evolved repeatedly across the species range that inhabit contrasting habitats on the shore (Panova *et al*. 2006; Butlin *et al*. 2014). Local adaptation on a fine spatial scale is enabled by low dispersal as a result of the species lacking a pelagic larval stage (Reid 1996). In Sweden, two main ecotypes exist: 'Crab' and 'Wave', named after the principal selection pressure in the areas they are found (Johannesson *et al*. 2010). The Crab ecotype inhabits boulder fields where they experience crab predation, so have adapted large, thick shells with small apertures and wary behaviour (Johannesson *et al*. 2010). In contrast, the Wave ecotype is found on rocky headlands where wave action is the predominant selection pressure; they are small with relatively large apertures to better anchor to the substrate and enable sheltering in small crevices in the cliff (Johannesson *et al*. 2010). The two ecotypes successfully hybridise and form hybrid zones at the habitat transition between the two environments where reproductive isolation is low, although assortative mating through size-based mate choice does occur (Johannesson *et al*. 1995; Perini *et al*. 2020). More recently, polymorphic inversions across multiple chromosomes have been identified in *L. saxatilis* that are thought to be involved in local adaptation (Westram *et al*. 2018; Faria *et al*. 2019; Morales *et al*. 2019; Koch *et al*. 2021; Westram *et al*. 2021). This includes inversions on a linkage group that holds an SD locus that are differently associated with sex in each ecotype (Koch *et al*. 2021; Hearn *et al*. 2022; Koch *et al*. 2022).

Transplant experiments are useful tools for testing for both divergent selection and habitat choice and their impact on local adaptation with gene flow (Webster *et al*. 2012). They have been employed in other *Littorina* species (Antwi and Ameyaw-Akumfi 1987; Chapman 1999; Miller *et al*. 2007) in addition to Swedish and Spanish populations of *L. saxatilis* (Janson 1983; Erlandsson *et al*. 1998; Cruz *et al*. 2004). In Sweden, ecotypes within their native habitat dispersed smaller distances and were recaptured more often than the other ecotype transplanted into that environment, suggesting habitat choice and/or differential survival (Janson 1983). Experiments on Spanish shores concluded that both differential movement and survival of transplanted snails contributed to the reformation of a phenotypic cline; transplanted snails moved greater distances and more directionally, and lower viability was implied through decreased recapture rates (Erlandsson *et al*. 1998; Cruz *et al*. 2004). The experiments also gave an estimated dispersal rate for *L. saxatilis* of up to two metres per month (Janson 1983; Erlandsson *et al*. 1998). Cruz *et al*. (2004) suggest that differences in survival between ecotypes across habitats (i.e. divergent selection) are more important than habitat choice in maintaining differentiation; in concurrence, lack of habitat preference in hybrids supports the role of habitat-based selection as a primary driver (Carballo *et al*. 2005).

However, the difficulties in untangling differential survival and dispersal between ecotypes in these kinds of experiments mean that results are uncertain and questions remain. Further, quantitative estimates of ecotype-habitat interactions in parameters such as survival and dispersal are likely to be useful for other studies but were not calculated (Webster *et al*. 2012). Years of study on hybrid zones and new knowledge of locally adaptive inversions, sex determination and sex-linked inversions in *L. saxatilis* since the last transplant experiment of this nature highlights a significant opportunity for an updated study.

Therefore, we conducted a large-scale reciprocal transplant mark-recapture experiment replicated across two populations of Swedish *L. saxatilis*. Snails from across the shore- Crab and Wave habitats and the hybrid zone- were included in order to study differences in

phenotype, genotype, movement, survival, and their interactions. Specifically, we aimed to test for evidence of habitat choice and habitat-based divergent selection, and the association of inversions with these, to help understand their roles in local adaptation. We additionally investigated sex-biased divergent selection on inversions implicated with an SD locus to help uncover the role of sex differences and sex-linked inversions in the adaptive divergence process.

Locally-adaptive phenotypic differences between ecotypes are well-characterised, and divergent selection pressures across the environments were therefore expected to result in better survival of transplanted snails that were closer in phenotype to the native ecotype in each environment. For example, of the Crab snails transplanted into Wave habitat, the smaller, lighter individuals were expected to be more successful. Accordingly, significant effects of ecotype, release habitat, recapture success, and their interactions on weight and thickness were anticipated. One might also expect slower growth in ecotypes in non-native than native habitats due to being transplanted into a sub-optimal environment.

The studied inversions were chosen for their frequency differences and proposed role in divergent selection between ecotypes. Therefore, inversion frequency differences were expected between ecotypes and release habitats due to differential survival across habitats based on inversion genotypes, with frequencies in an ecotype in a non-native habitat shifting towards that of the native ecotype in that habitat. Additional sex effects are expected for the sex-linked inversions, but the complex nature of their sex-ecotype-inversion associations and unknown genetic mechanism of sex determination mean that specific predictions are equivocal. One may expect, though, that sex differences in inversion frequency in Wave or hybrid snails may appear in those transplanted to Crab habitats through sex-specific survival differences of individuals of different genotypes.

Movement metrics were used to test predictions of habitat choice. In general, all metrics were expected to vary with ecotype and release habitat in an interactive manner to result in movement of transplanted snails back towards their native environments. Snails in non-native habitats should move greater distances and more directionally than those in their native habitat.

Divergent selection across environments was expected to become apparent through differential survival probabilities between groups. Ecotypes were expected to have the highest survival probabilities in their native habitat compared to non-native habitats, and within each habitat, the native ecotype was expected to have the highest survival. Across the hybrid zone, it was predicted that Crab and Wave ecotypes should have better survival in the Hybrid habitat relative to survival of the Hybrid ecotype in Crab or Wave habitats. The strength of the main selection pressures (wave action and crab predation) change over time; therefore, it was predicted that survival would change over time, and in the relevant habitat they would track the variation in selection pressure. Encounter probability was likely to vary with ecotype and habitat due to differences in ease of searching during fieldwork.

4.3 Methods

Field sampling and sample processing

Fieldwork was carried out at two sites on separate islands on the west coast of Sweden- Ängklåvebukten (58°52’15.14”N 11°07’11.88”E) and Inre Arsklovet (58°50'00.5"N 11°08'19.6"E), referred to as ANG and CZB, respectively, from here on (Figure 1). These sites are located close to the University of Gothenburg marine research laboratory on the island of Tjärnö, which was used as a base and for lab work. The sites were selected as they have previously been used in various *L. saxatilis* studies (Westram *et al*. 2018, 2021), enabling utilisation of current knowledge to inform experimental design. The same experimental procedure was carried out at both sites.

The study followed a mark-recapture methodology, with reciprocal transplant between three areas: the two ecotype habitats (Crab and Wave) and the hybrid zone (Figure 1). The regions of Crab and Wave habitat selected were characteristic habitat away from the hybrid zone used in previous studies- so that ‘pure’ ecotypes, genetically and phenotypically, were used. From here on, hybrid zone snails and the hybrid zone are often referred to as the ‘Hybrid ecotype’ and ‘Hybrid habitat’ for simplicity, but in reality are intermediates between the two ecotypes and environments. Since part of the experiment aimed to test genotype frequency changes of focal inversions, it was important to use the area of hybrid zone at which inversion frequencies were at their most intermediate. Equal inversion genotype frequencies at release would give

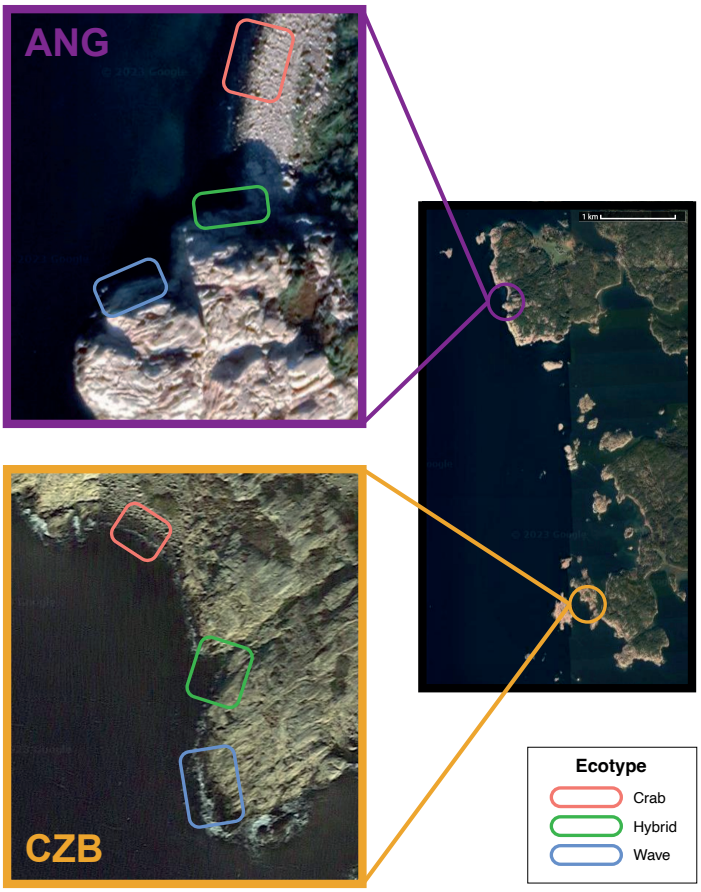


Figure 1. Satellite images of the two study sites and the location of the two islands- ANG and CZB- in relation to one another. Coloured rectangles within sites indicate the approximate locations of the areas of habitat for the three ecotypes used in the experiment. Satellite images taken from Google Maps.

the most power to detect selection on each inversion genotype, with equal power for each genotype. To achieve this, the mean centre was found, across clines for all inversions to be investigated in this study. Cline parameters used were those calculated in Westram *et al*. (2018) and Westram *et al*. (2021) and represent a distance on the transect path fitted to the samples in those studies. The mean centre value was transformed back from a transect position into coordinates that could be used to locate the position on the shore itself. The centre of the hybrid zone in both sites had a rock substrate, as in the Wave habitat (Figure 1), even though other environmental variables were likely to be intermediate- especially wave exposure and crab predation (although the rock was quite vertical in the ANG hybrid zone which may reduce the presence of crabs).

Snails were handled in sets of 96 individuals throughout the experiment. As many sets were released as was allowed by weather and time constraints during fieldwork periods. To minimise the time that snails were kept in the lab between collection and release, collections were only carried out when suitable weather conditions were also forecast for the following days. A small number of sets to be released consisted of snails reared in the lab rather than collected from the field. These were recombinant F_X snails from lab families originally founded by Crab x Wave crosses. The majority of fieldwork was carried out across three three-week periods in 2019: April-May, July, and September. Releases took place in all periods whereas recaptures were carried out from July onwards. Final recaptures were additionally carried out over three single days in October.

Collection:
One collection, comprising one set of snails, was carried out at a time with the next set not collected until the previous set had been released. Each set of 96 comprised 32 snails (one third) from each of the three habitats. A small number of spare snails were collected in each habitat in case of species misidentification in the field or problems during processing. Hybrid zone snails were collected from the calculated inversion cline centre; Crab and Wave snails from their characteristic habitats (Figure 1). The position of the centre of the collection area for each habitat was measured in three dimensions using a Trimble total station. Collection was within a few metres from this point. The same area was used for collection for each set so this position was only taken during the first collection round at each site. To ensure ability to label, only snails of a greater length than 3mm were collected.

Phenotyping and marking:
Processing took place in the lab on the same day or day after collection, during which snails were stored at 4°C. Each snail was phenotyped for a number of traits before the marking procedure. Photos were taken in three predefined orientations for use in morphometrics, using a camera, microscope, and PC imaging software. Shell thickness was recorded in three locations around the aperture using a digital dial indicator, in addition to the colour category and level of scarring of the shell. Snails were also weighed.

Snails were then labelled. Labels consisted of a 3-digit alphanumeric code- a letter followed by the numbers 01-96, with a different letter used for each set of snails- printed onto waterproof paper then cut out. Gorilla glue was used to adhere the label to the snail. Gluing was most effective when shells were dry. Once the glue was dry, a thin layer of nail varnish was painted around the outside edge of the aperture; this allowed measurement of shell growth upon recapture.

Release:
Release was done as soon as possible after processing and drying of the labels. In the lab, snails were split into numbered release groups. Snails from each of the three habitats were split in a ratio of 1:2:1 for release into the three habitats, such that twice as many would be released into the hybrid zone as each of Crab and Wave. Each ecotype was therefore released both back into its own habitat and the two alternate habitats. Releases occurred as groups of 8 individuals so that not all snails were released at the same point. In total this gave three release groups (Crab, Wave, hybrid) in each of Crab and Wave habitats and double in the hybrid zone (two groups each of Crab, Wave, hybrid). The precise point of release of each group was recorded, again using the total station. Release points were all located in the areas around the collection points; other *L. saxatilis* individuals must have been present around the release points to ensure that it was suitable habitat for the snails.

Short-term movement:
A short period after release, usually a day or two depending on weather, short-term movement was recorded. This was carried out through searching the shore for any marked individuals and use of the total station to record the new positions of all snails found and date of recording. Care was taken to read the code on the label without picking up or dislodging the snail from the position where it was found. Positions were taken for all marked snails found during searching regardless of their set; therefore, movements of some snails were recorded multiple times in the weeks after their release when they were located again during searching after later sets of releases.

Recapture:
Recapture of snails occurred after a few months to allow time for longer-term movement and selection. Only marked individuals that were released in a previous fieldwork period were recaptured when found on the shore, with instead a short-term movement record (as above) taken for any snails released in the current period. Since each release set used a different letter in the label code, these could be used to identify the time of release. The precise positions of snails to be recaptured were recorded with the total station before taking for processing in the lab. Date of recapture was also recorded.

Photographs in the same three orientations were taken as prior to release. Growth was measured under a light microscope as the distance from the nail varnish mark to the new aperture margin, following around the centre of the last whorl. Finally, snails were dissected; presence of penis or brood pouch was recorded (or neither, indicating a juvenile) and head and foot tissue preserved in ethanol for later DNA extraction.

Genotyping
DNA extraction and SNP genotyping via KASP was carried out by LGC from stored tissue. A set of 80 SNPs was selected from SNP sets used in previous *L. saxatilis* studies. They were selected on the basis of being informative for indicating inversion arrangements for focal inversions on linkage groups (LG) 6, 14 and 17. The set also included sex-associated SNPs on LG12 and controls in collinear regions on these linkage groups. The distribution of SNPs across inversion and collinear regions on each linkage group is shown in Table 1. To enable primer design, selected SNPs were required to have minimal polymorphism in the 50bp on either side of the SNP, with a maximum minor allele frequency of 0.05 for any non-focal SNPs

Table 1. The number of SNPs that were successfully genotyped for each inversion and collinear region of each linkage group. LG12 has no collinear region as the four inversions cover the entire linkage group.

Linkage group	Region of linkage group	Number of SNPs
6	Collinear	7
	LGC6.1	4
	LGC6.2	5
12	LGC12.1	8
	LGC12.2	23
	LGC12.3	6
	LGC12.4	4
14	Collinear	3
	LGC14.1	4
	LGC14.2	4
	LGC14.3	3
17	Collinear	5
	LGC17.1	4

that were present in the 50bp region. SNPs were selected as informative for inversion arrangements through high differentiation between Crab and Wave, with a strong association between genotype and inversion arrangement. Informative SNPs for LG12 were chosen based on the heterozygosity pattern that is indicative of the association with sex previously characterised (Chapter 2)- those with a heterozygote proportion of near 1 in females and near 0 in males in Crab, but equal proportions between sexes in Wave.

It is now known that LG12 is involved in sex-determination; it is covered by four inversions that show ecotype differentiation, of which some also show sex-association (Hearn *et al*. 2022). This had not yet been identified when SNP selection was carried out; however, as the sex-associated and collinear SNPs span LG12, inversion genotyping of the four LG12 inversions was still possible. There was a much higher number of SNPs in the LGC12.2 inversion region than elsewhere (Table 1) as the majority of sex-associated SNPs are in located in that region.

Analyses

All analyses were carried out in R (version 4.0.0; R Core Team 2020) and used the packages DPLYR (version 1.0.5; Wickham *et al*. 2021) and GGPLOT2 (version 3.3.0; Wickham 2016) unless otherwise stated.

Data-processing:

Before analyses, the various forms of data were cross-checked to ensure no mislabelling of snails had occurred at any point. This included the total station position records for release, movement and recapture, release and recapture photos, and dissection records. Corrected data were then compiled by snail ID. A small number of snails were recaptured that had lost their labels but had retained the nail varnish marker; as these could not be matched to their snail data, they were excluded from all analyses. F_X snails were excluded from all analyses

(aside calculation of recapture rate) to enable testing of wild ecotype differences, as it was clear that the F_X snails behaved quite differently to all others.

Phenotypes:
Weight and shell thickness data for all snails were available as they were phenotyped before release, which enabled comparisons between recaptured and non-recaptured snails across groups. Shell growth was available for recaptured snails only. The three phenotypes were tested using linear models followed by ANOVAs to test significance. Weight and thickness were highly correlated with one another (r^2=0.74) so a thickness measurement independent of weight was calculated by dividing thickness by weight; hereafter referred to as thickness for simplicity. As snails were on the shore for different lengths of time before recapture, growth measurements were standardised by dividing by the number of days between release and recapture. The distributions of all three phenotypes were right-skewed, so were log-transformed prior to model fitting. Main and interactive effects of ecotype, release habitat and recapture success (recaptured vs not recaptured) on weight and thickness, and ecotype and release habitat for growth, were tested in the models.

Survival and recapture probabilities:
The program Mark (version 10.x; White and Burnham 1999) was used, with its RMARK (version 3.0.0; Laake 2013) R interface. Data for all snails, whether recaptured or not, were included in this analysis. Total station data gave records of the dates that snails were released, sighted (movement records), and recaptured. These were transformed into the encounter history format required by Mark. Recaptured snails were recorded as 'removals', denoted by -1 in the frequency column, to differentiate from snails with movement data that were not recaptured. Grouping variables of ecotype (Crab, Wave, Hybrid) and release habitat (Crab, Wave, Hybrid) were also added. Time intervals between occasions were specified in days, and a standard CJS (Cormack-Jolly-Seber) model for live encounters was used. On a few occasions, snails were released or collected without searching for movement or recaptures; therefore, the recapture probability parameter was fixed in the design data to be zero for those dates.

Three additional covariates were prepared for use in later models. Weight and thickness were collected as phenotypic data (as above) and were added as individual covariates to the

Table 2. Which parameters (Phi (ϕ) and/or *p*) were being tested in each model set, and which datasets these models were tested on (the full dataset and/or the ecotype and release habitat subsets). X denotes yes- the parameter was being tested/the dataset was tested. Where a parameter was not tested, the formula used in all models in that set is given.

Model set	Varying formulae? If no, which formula set		Datasets tested	
	ϕ	*p*	Full	Subsets
A(1-5)	X	~1	X	X
B	~ ecotype * release_habitat + time	X	X	
C	X	X	X	
D1	X	~ ecotype * release_habitat + time	X	
D2	X	X		X

Table 3. The variables included in ϕ and/or *p* formulae tested in each model set- where X denotes that the variable was included. Where all variables are blank for ϕ or *p* for a model set, that parameter was not being tested so a constant formula was used- see Table 2. All variables and their interactions (aside time interactions) were combined factorially within ϕ and *p* to produce the formulae for testing.

Model set	ϕ						*p*		
	Ecotype	Release habitat	Time	Weight	Thickness	Waves	Ecotype	Release habitat	Time
A1	X*	X*	X						
A2	X*	X*	X	X					
A3	X*	X*	X		X				
A4	X*	X*	X			X			
A5	X*	X*	X	X	X	X			
B							X	X	X
C	X	X	X				X	X	X
D1	X	X	X	X	X	X			
D2	X*	X*	X	X	X	X	X*	X*	X

** denotes variables that were included in the full dataset analysis formulae for that model set, but excluded from formulae tested in the subsets (ecotype or release habitat sets) where not necessary.*

Table 4. ϕ and *p* formulae tested in the non-factorial model sets (D1 and D2). Each *p* formula is tested with each ϕ formula in model testing. In D2, the different formulae required for the ecotype and release habitat data subsets are given.

Model set	ϕ formulae	*p* formulae
D1	~ 1	~ ecotype * rel_hab + time
	~ time	
	~ ecotype	
	~ rel_hab	
	~ ecotype + time	
	~ rel_hab + time	
	~ ecotype + rel_hab	
	~ ecotype * rel_hab	
	~ ecotype + rel_hab + time	
	~ ecotype * rel_hab + time	
	~ ecotype * rel_hab + time + weight	
	~ ecotype * rel_hab + time + thick	
	~ ecotype * rel_hab + time + PC1	
	~ ecotype * rel_hab + time + weight + thick	
	~ ecotype * rel_hab + time + weight + PC1	

	~ ecotype * rel_hab + time + thick + PC1 ~ ecotype * rel_hab + time + weight + thick + PC1	
D2 (ecotype subsets)	~ 1 ~ time ~ rel_hab ~ rel_hab + time ~ rel_hab + time + weight ~ rel_hab + time + thick ~ rel_hab + time + PC1 ~ rel_hab + time + weight + thick ~ rel_hab + time + weight + PC1 ~ rel_hab + time + thick + PC1 ~ rel_hab + time + weight + thick +PC1	~ 1 ~ time ~ rel_hab ~ rel_hab + time
D2 (release habitat subsets)	~ 1 ~ time ~ ecotype ~ ecotype + time ~ ecotype + time + weight ~ ecotype + time + thick ~ ecotype + time + PC1 ~ ecotype + time + weight + thick ~ ecotype + time + weight + PC1 ~ ecotype + time + thick + PC1 ~ ecotype + time + weight + thick +PC1	~ 1 ~ time ~ ecotype ~ ecotype + time

Abbreviations: Thick = thickness; Rel_hab = release habitat; PC1 = waves PC1

capture histories. Waves- selected as the aspect of weather most likely to affect survival- was added as a design covariate to the design data. The wave data was downloaded from the Swedish Meteorological Institute (SMHI; open access under Creative Commons Attribution 4.0; *smhi.se/en*) and contained five parameters of wave action that were summarised at hourly intervals- sign wave height; maximum wave height; average wave period; maximum wave period; and compass direction. To merge with the design data, one value was required per time interval in the data (i.e., number of days between each visit to the site) so summary statistics (mean- sign wave height, average wave period, compass direction- or maximum- max wave height, max wave period) were calculated for each variable for each time interval.

To produce a single variable that encompassed all four measures of 'waviness' for use in RMark, principal component analysis (PCA) was then carried out on the four variables (excluding compass direction). Plotting showed that each of the four variables increased linearly with PC1, indicating that PC1 did indeed predict 'waviness' and was suitable for use in RMark. Plotting PC1 against compass direction showed that as PC1 increased (indicating more wavy conditions), the compass direction became closer to 240 degrees, whilst at low values of PC1, the compass direction was variable. As wavy conditions were all produced from a Westerly wind, as expected, and the aspect of our sites also faced this direction, it was

not deemed necessarily to transform the circular compass direction into linear variables or include those in the PCA, as PC1 already effectively represented wind direction.

Two parameters can be estimated in Mark: phi (ϕ)- the apparent survival probability (between occasions), and *p*- the recapture probability (on each occasion). In this experiment, a simple 'recapture rate' was also estimated as the proportion of snails that were released that were recaptured; therefore, to avoid confusion, the RMark recapture probability will be referred to as the 'encounter' rate or probability. The focal interest of this analysis was survival (ϕ). However, encounter probability was also included in testing as it may vary across groups and time, so needed to be accounted for in models. Sets of variables were tested sequentially before building up to more complex models (Tables 2-4). This was done to determine relevant variables to include in the more complex models, as inclusion of all parameter and interaction combinations would have resulted in very high computational load and long run times. Time was included in all sets as it was likely that survival would vary temporally across the experiment period (April-October). Sets of models were run on subsets of the data in addition to the full dataset (Table 2): snails were split into separate ecotypes or separate release habitats, giving 6 subsets (F_X snails were excluded due to a very low recapture rate).

First, *p* was set to constant (*p*~1) while the effect of ecotype and release habitat on ϕ was tested (model set A1; Tables 2-3). The three additional covariates- weight, thickness, and waves- were then added to these sets of models individually for testing (model sets A2-4; Tables 2-3), before testing them simultaneously in a more complex set of models (model set A5; Tables 2-3). In all model sets, the two-way interactions between all parameters apart from time were also tested, making it an almost factorial design. These were run on the full dataset as well as on separate ecotype and separate release habitat datasets (so ecotype or release habitat predictor variables were dropped where not relevant).

To test whether encounter probability did vary across groups or over time, ϕ was set to one formula (~ecotype * release_habitat + time, as these variables were consistently important in the above testing) while testing models with variation in *p*. Combinations of ecotype, release habitat and time plus their interactions (factorial, aside from time interactions) were tested for *p* on the full dataset (model set B; Tables 2-3). As the best model was not *p*~1 (constant), both *p* and ϕ were then tested together with factorial (aside time interactions) combinations of ecotype, release habitat, time, and interactions for each (model set C; Tables 2-3). The best models consistently had non-constant formulae for both *p* and ϕ; therefore, the best *p* formula (~ecotype * release_habitat + time) was included in the final analyses of ϕ where all variables (ecotype, release habitat, time, weight, thickness, waves) were tested in the complex set of models (model set D1; Table 2 and Table 4). In the analyses with a constant *p*, all top models for ϕ included ecotype, release habitat, and time. Therefore, this time a smaller selection of ϕ formulae was tested, rather than the fully factorial list, to exclude unnecessary combinations and reduce the time taken to run. Finally, the complex model set was tested in the separate ecotype and release habitat datasets; this time with *p* formulae also included (model set D2; Table 2 and Table 4).

In all sets tested, following model fitting, models for each dataset were compared. Models were ranked according to AICc value and ΔAICc to the best fitting model. Models were then averaged, weighted by AICc, for both ϕ and *p*, with the drop=FALSE argument included to

ensure models with non-positive variances for betas were not dropped (Laake and Rexstad 2008). The real estimates of ϕ and *p* from the averaged model were extracted for each dataset.

Genotypes:
Only recaptured snails could be genotyped so analyses were focussed on differences between groups of recaptured snails, rather than the change from release to recapture. First, snails were genotyped for inversions based on the SNP genotypes. As in previous studies of *L. saxatilis*, principal component analysis (PCA) was employed. This was carried out using the packages HMISC (version 4.6.0; Harrell Jr and Others 2020) and ADEGENET (version 2.1.3; Jombart 2008; Jombart and Ahmed 2011). PCA was carried out on SNPs in each inversion region. If the three characteristic clusters denoting the three inversion genotypes (the two homokaryotypes with the heterokaryotype cluster in between) were present, genotypes were simply assigned according to the clusters. Individuals were also labelled by their heterozygosity count (number of heterozygous SNPs per snail) to help confirm cluster identity. If PCA clusters were not clear, SNP genotypes for that region were checked. Any SNPs that were not informative- showed little variation or clearly were not in LD with the others- were removed and the PCA repeated. Genotyping was done from these PCA clusters in conjunction with examination of the SNP genotypes. For the two complex inversions on LG6 and LG14, if six clear clusters were not present in the PCA, the inversions were instead genotyped for only the two main haplotypes- by three PCA clusters- since the third haplotype is low frequency and not as informative.

For those inversions that could be genotyped, inversion genotype and arrangement frequencies were calculated separately for each ecotype and release habitat combination in each site. Differences in inversion frequencies between ecotypes and release habitats were tested using generalised linear models (glm), with the counts of each arrangement per group as the response variable; ecotype, release habitat and their interaction as the predictor variables; and a binomial distribution with logit link. For the inversions on the linkage group associated with sex (LG12), sex and its interactions with the other predictor variables were also included in the models.

Movement:
Methods for studying movement ecology are extremely varied and depend greatly on the type of data collected (often limited by the studied species) and the goal of the analysis (Nathan *et al.* 2008; Hooten *et al.* 2017); therefore, many of these were not possible or relevant to this study. Movement analyses testing habitat selection, i.e. linking animal movement to their environment, typically use habitat-selection or step-selection functions and complex models (Thurfjell *et al.* 2014; Fieberg *et al*. 2021; Mercker *et al*. 2021). These require position data that is continuous or with small time intervals, and environmental sampling of a range of abiotic and biotic habitat variables over space and time in parallel to the movement recordings (Thurfjell *et al*. 2014; Seidel *et al*. 2018). However, here it was possible only to collect individual-based point observation data with coarse-scale time intervals. While limited by the data collected, the above methods were also testing more complex aspects of resource use and habitat selection than required here (Fieberg *et al.* 2021; Mercker *et al*. 2021). The linear distribution of ecotype habitats in a narrow band along the shoreline allowed a further simplified approach: it was possible to separate directional aspects of movement relative to environmental features (i.e. the sea and other ecotype habitats) directly from pairs of point coordinates. Both the directional and non-directional distance metrics used here were

therefore able to be tested with combinations of simple displacement calculations (Turchin 1998; Seidel *et al.* 2018) and linear models.

Total station data, i.e. X, Y and Z spatial Cartesian coordinates, were used for the movement analysis; any snails with more than one position (i.e. more than just the release position) could be included. A pair of consecutive position records for an individual denotes one movement. Positions were used to calculate a number of variables encompassing aspects of movement for each pair of positions: along-shore movement, seaward movement, vertical movement, and overall distance moved. Totals for each individual and scaled (per-day) values were then calculated. The overall distance of travel for each snail was summarised by two measures: distance from release was the straight-line distance between the first (release) and final (movement or recapture) position recorded for a snail, and the total distance moved was the sum of all straight-line distances between consecutive positions for a snail. If a snail only had two recorded positions these two measures would therefore be the same. These two variables were calculated using the formula for distance between two points (where $P_1 = (x_1, y_1, z_1)$ and $P_2 = (x_2, y_2, z_2)$) in xyz-space:

$$d(P_1, P_2) = \sqrt{(x_2 - x_1) + (y_2 - y_1) + (z_2 - z_1)^2}.$$

Eqn.1

Total distance moved gave a more general estimate of activity and distance from release might have suggested less random movement. Specifically, a close to 1:1 ratio between the two metrics implied directional movement away from release whereas a much larger total distance moved than distance from release implied random movement around the area.

Vertical movement was simply the difference in Z coordinates between the two points, with positive values corresponding to an upward movement and vice versa for negative. Along-shore and seaward movement are the aspects of movement in relation to the shoreline: along-shore is movement parallel to the water's edge (i.e., along the transect and between habitats) and seaward is movement perpendicular to the water's edge (i.e., towards or away from the water). These were calculated separately for each habitat at each site as the angle of the shore varied across the bays. Positions were rotated around the origin (0,0) until the shoreline became parallel to the X axis, upon which along-shore movement became equal to the difference in X coordinates between the two positions (where positive values correspond to moving to the right when facing the shore from sea (Crab habitat is on the left)), and seaward movement became equal to the difference in Y coordinates (where positive values correspond to movement away from the water).

Movement was analysed using linear models. Total movement values, scaled by time to per-day values, were used per snail for each of the variables. Distance moved and distance from release were both log-transformed to account for the right-skew. Ecotype, release habitat and recapture success (yes vs no), and their interactions, were tested as predictor variables. ANOVA was then performed on the linear models to test significance.

4.4 Results

Table 5. Numbers of snails recaptured of each ecotype and sex from in each release habitat, at both sites ANG and CZB. F_x lab snails are excluded.

		Ecotype									
		Crab			Hybrid			Wave			
		F	M	u	F	M	u	F	M	u	Totals
ANG	Crab	8	12		9	9	1	4	5		48
	Hybrid	12	6	1	25	13	2	14	7		80
	Wave	7	4		16	4		9	6		46
Totals		27	22	1	50	26	3	27	18		174
	Crab	10	10	1	13	17	3	6	2	1	63
CZB	Hybrid	23	14	1	21	21	3	30	13		126
	Wave	10	2	2	13	7		15	5		54
Totals		43	26	4	47	45	6	51	20	1	243

Abbreviations: F=female; M=male; u=unknown (juvenile or not recorded)

Across the two sites, 1438 snails were released in total - 672 at ANG and 766 at CZB. By the end of the experimental period, 174 snails were recaptured at ANG and 243 at CZB, equating to a recapture rate of 25.9% and 31.7% respectively. Table 5 gives the breakdown of recaptured snails across sex, ecotype, and release habitat groups. The sex ratio of recaptured snails was around 1.6 females per male in both sites. F_x snails from the lab were distinct from all wild ecotypes as they suffered a vastly decreased recapture rate; in ANG, only three individuals were recaptured and twelve in CZB, giving recapture rates of 6% and 13%, respectively (Figure 2). As a result of the small sample size, F_x snails were excluded from all analyses.

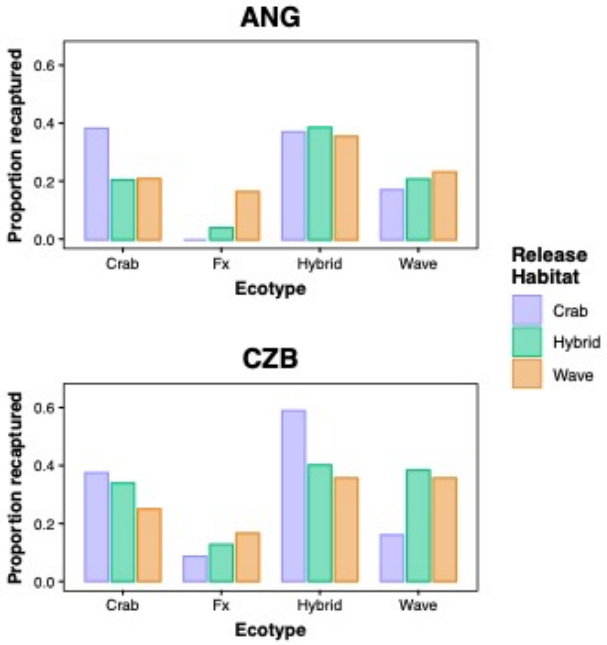


Figure 2. The proportion of each ecotype released in each habitat at each site that were recaptured.

Phenotypes

Weight and relative shell thickness (weight-controlled) of all snails were measured before release, while shell growth of recaptured snails was measured. Divergent selection across habitats was expected to result in better survival and recapture of snails that were closer in phenotype to the native ecotype in each environment, producing significant interactive effects of ecotype, release habitat, and recapture success on phenotypes.

Ecotype differences in weight and thickness were confirmed in both ANG (weight $F=368.9$, $df=2$, $p<0.001$; thickness $F=100.4$, $df=2$, $p<0.001$) and CZB (weight $F=268.2$, $df=2$, $p<0.001$; thickness $F=95.9$, $df=2$, $p<0.001$; Figure 3; Supplementary Table 1). Crab individuals were the heaviest, followed by Hybrid, then Wave; however, thickness showed the opposite trend (probably due to dividing by weight). In ANG, weight further differed with release habitat ($F=3.1$, $df=2$, $p<0.05$) and recapture success ($F=6.4$, $df=1$, $p<0.05$; Figure 3), although there were no significant interactions. Recaptured snails were heavier than non-recaptured snails, but by a much smaller magnitude than the variation between ecotypes; differences in weight between release habitats were on a smaller scale again than both of the previous group types. CZB snails followed the same trend in weight differences between recaptured and non-recaptured snails ($F=7.6$, $df=1$, $p<0.01$; Figure 3). Testing of each release habitat separately showed that the effect of recapture success was significant in the Crab release habitat only, in both sites (ANG: $F=5.7$, $df=1$, $p<0.05$; CZB: $F=3.0$, $df=1$, $p<0.01$). In CZB, when considering each ecotype individually, recaptured snails of the Wave ecotype were slightly larger than non-recaptured snails ($F=8.0$, $df=1$, $p<0.01$). Thus, the central prediction of significant interactions

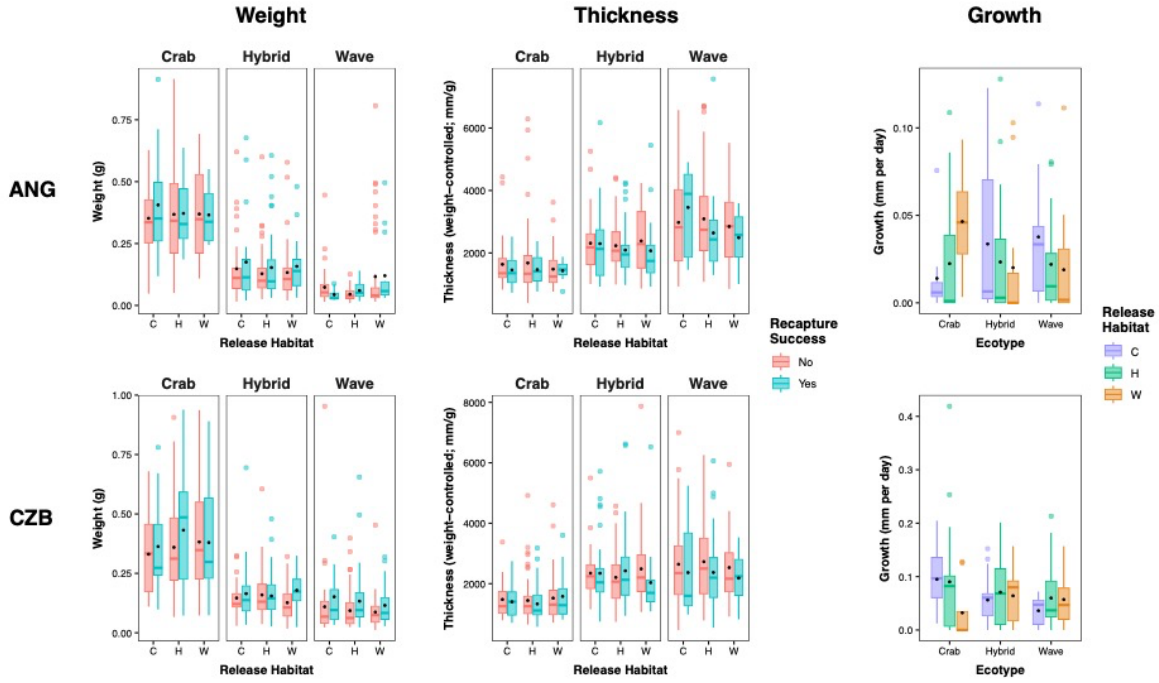


Figure 3. The weight and the weight-controlled shell thickness (thickness divided by weight) of all released snails, separated by whether they were recaptured in the experiment, per ecotype and release habitat for each site. The shell growth of recaptured snails from release to recapture, per ecotype and release habitat for each site. Black points indicate mean values for each group.

between predictor variables (ecotype*habitat*recapture success, ecotype*recapture success, or habitat*recapture success, depending on the dataset) was not met for weight or thickness in either site.

Loss of nail varnish from the shell while snails were on the shore led to a reduced sample size for the growth analysis. Varnish was lost from 21% of ANG and 33% of CZB recaptured snails. Trends appeared inconsistent between the two sites, and no overall differences between ecotypes or release habitats were present (Figure 3; Supplementary Table 1).

Genotypes

All inversions aside from LGC12.1 were successfully genotyped at both sites, although the complex inversions on LG6 and LG14 could only be genotyped for two of the three haplotypes. For a few inversions, a small number of snails were intermediate between PCA clusters and could not be assigned genotypes. It should be noted that the numbers of individuals in some ecotype/release habitat/sex groups were small (Table 5) so arrangement frequencies may be unreliable. Previous research could not find evidence for LGC12.3 in the CZB population (Chapter Three). In contrast, CZB snails in this study seemed to indicate presence of LGC12.3 and were genotyped as such. This may be a product of low sample size so any results involving LGC12.3 should be interpreted with caution.

The inversions studied were selected based on their proposed role in divergent selection and previously documented frequency differences between ecotypes (Westram *et al.* 2018, 2021; Hearn *et al.* 2022). Therefore, differential survival was expected across habitats based on inversion genotype to produce different frequencies across transplant habitats within each ecotype. Different sex-, ecotype-, and habitat-specific survival of each genotype of the sex-linked inversions was likely to produce complex patterns of frequency differences.

Arrangement frequency was highly associated with ecotype for all inversions at all sites (see Table 6 for significance values; Supplementary Table 2), and patterns were generally consistent between ANG and CZB (Figure 4a-b). As would be expected, arrangement frequency in hybrid individuals was almost always in between frequencies in Crab and Wave.

Table 6. The significance of the effect of ecotype on arrangement frequency for all inversions at both sites. Tests were carried out using generalised linear models followed by ANOVA. F_x snails were excluded from tests.

	ANG			CZB		
	χ^2	df	p	χ^2	df	p
LGC6.1	15.9	2	<0.001	39.2	2	<0.001
LGC6.2	16.9	2	<0.001	38.0	2	<0.001
LGC12.2	18.7	2	<0.001	27.7	2	<0.001
LGC12.3	18.0	2	<0.001	29.8	2	<0.001
LGC12.4	32.3	2	<0.001	40.4	2	<0.001
LGC14.1	32.8	2	<0.001	133.6	2	<0.001
LGC14.2	21.5	2	<0.001	8.5	2	<0.05
LGC14.3	25.4	2	<0.001	28.6	2	<0.001
LGC17.1	65.9	2	<0.001	29.1	2	<0.001

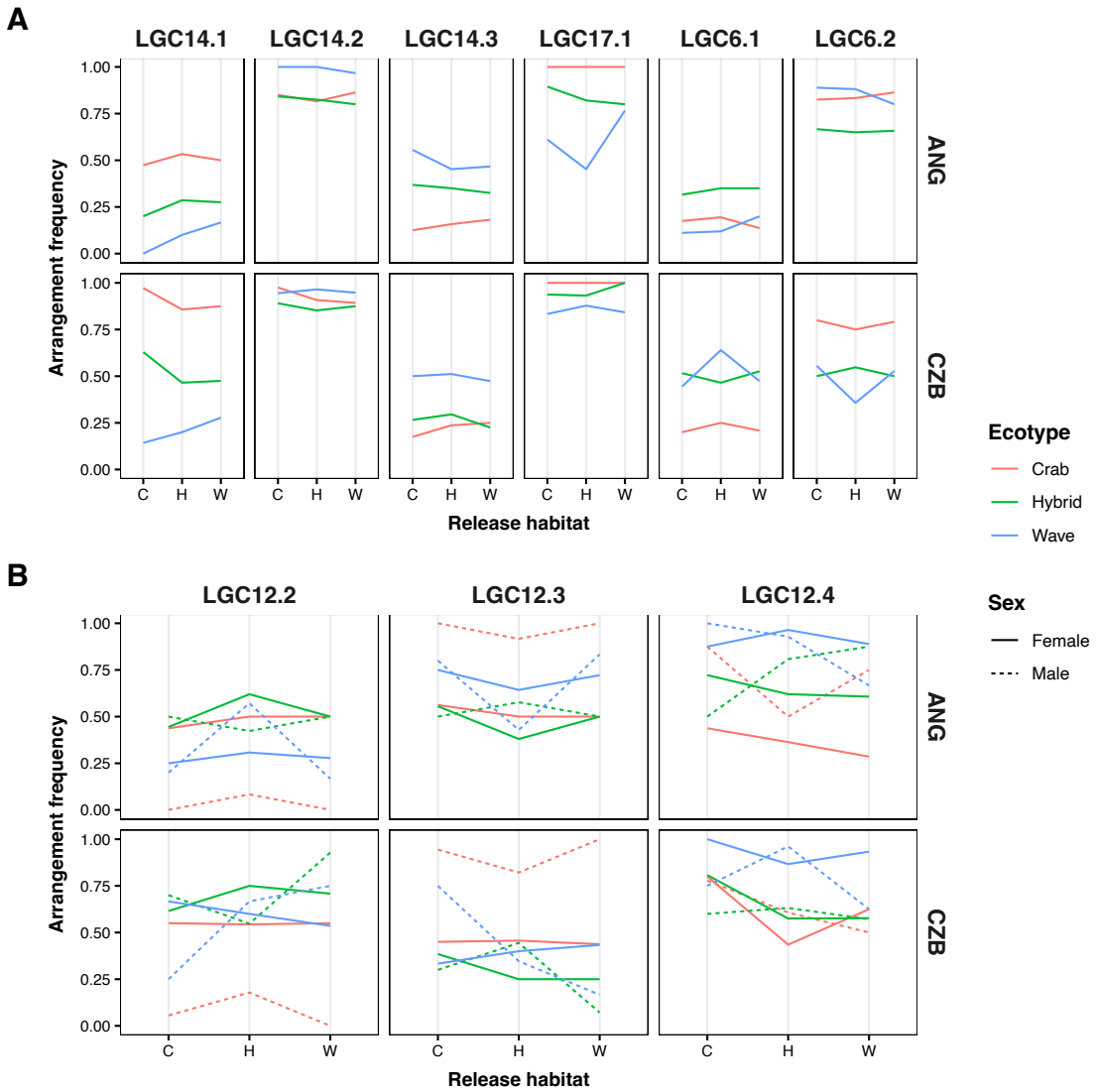


Figure 4. The arrangement frequency of **A)** non-sex linked and **B)** sex-linked inversions in recaptured snails in each ecotype and release habitat (and sex in **B**) group. Each line connects the frequencies in each habitat for an ecotype.

However, there were no differences in arrangement frequency between release habitats and no ecotype-release habitat interactions (Supplementary Table 2). The only exception was for LGC12.4 in CZB- here, frequency differed between release habitats (χ^2=8.8, df=2, p<0.05). Within- ecotype tests showed that differences between habitats were present only in the Crab ecotype (χ^2=11.1, df=2, p<0.001), specifically between the Crab and other habitats where the arrangement frequency fell from 0.80 in Crab to 0.51 and 0.54 in Hybrid and Wave. This difference was also present within the Crab ecotype in ANG with a fall in frequency from 0.70 to 0.42 and 0.45 (χ^2=7.2, df=2, p<0.05), despite the lack of overall difference between release habitats across all ecotypes (χ^2=3.2, df=2, p<0.2). In ANG, the Wave ecotype also showed frequency differences of LGC17.1 arrangements, with a lower frequency in the Hybrid habitat- 0.45- than in either Crab- 0.61- or Wave- 0.77- habitats (χ^2=7.4, df=2, p<0.05). Lack of association in the other ecotypes produced an overall non-significant effect of release habitat (χ^2=4.2, df=2, p=0.31). Altogether, aside from the ecotype differences, few significant

differences in arrangement frequency were found between habitats and no ecotype*habitat interactions, and those that were significant should be treated with care due to multiple comparisons. The differences observed- between habitats for LGC12.4 within the Crab ecotype in both ANG and CZB and for LGC17.1 within the Wave ecotype in ANG- were generally messy but did not appear consistent with the expected direction of frequency change (Figure 4a).

The frequency of arrangements differed between the sexes for all three sex-linked inversions in ANG (LGC12.2: χ^2=8.4, df=1, p<0.01; LGC12.3: χ^2=9.1, df=1, p<0.01; LGC12.4: χ^2=6.1, df=1, p<0.05) and for two in CZB (LGC12.2: χ^2=7.4, df=1, p<0.01; LGC12.3: χ^2=7.3, df=1, p<0.01; Figure 4b; Supplementary Table 2). As expected, the relationship between sex and arrangement frequency also differed between ecotypes for these inversions in ANG (LGC12.2: χ^2=21.3, df=2, p<0.001; LGC12.3: χ^2=20.4, df=2, p<0.001; LGC12.4: χ^2=7.4, df=2, p<0.05) and CZB (LGC12.2: χ^2=18.9, df=2, p<0.001; LGC12.3: χ^2=18.5, df=2, p<0.001). Despite there being no LGC12.4 arrangement frequency difference between the sexes in CZB, frequency changes between release habitats varied between sexes (χ^2=7.4, df=2, p<0.05). Within-ecotype tests showed that this was driven by the Wave ecotype, where males displayed much greater frequency differences between release habitats than females and in the opposite direction. Further, a three-way interactive effect of ecotype, sex and release habitat on arrangement frequencies existed for LGC12.4 in ANG (χ^2=11.1, df=4, p<0.05). Sexes of all three ecotypes showed a differing association between frequency and release habitat. The frequency of LGC12.2 arrangements was also subject to this three-way interactive effect in CZB (χ^2=10.1, df=4, p<0.05). The frequency difference between release habitats was especially pronounced for males of the Wave ecotype, where the arrangement frequency rose from 0.25 in Crab to 0.67 in Hybrid and 0.75 in Wave.

Among the sex-linked inversions, the effect of multiple factors and their interactions on arrangement frequency were significant. Sex and sex*ecotype effects were detected across almost all inversions and sites, as expected for a sex-linked inversion. The most interesting predictions were those of habitat*ecotype*sex interactions (or habitat*sex within an ecotype dataset) and these were detected in a few tests: the interaction was significant for LGC12.2 in CZB and for LGC12.4 in ANG and the Wave ecotype in CZB. In these, males and females responded differently across habitats and this relationship varied between ecotypes for the two of three tests where ecotype was included (Figure 4b).

Movement

Snails that were sighted or recaptured at least once after release could be included in the movement analysis; this amounted to 64% of all snails at each site. In CZB, this was a roughly even split between snails that were and were not recaptured (243 vs 250). In ANG, it was biased towards snails that were not recaptured, i.e., with only movement recordings (171 vs 264). Most individuals had two or three positions (so one or two movements) recorded, but a few individuals had up to five or six positions recorded in ANG and CZB, respectively. A small number of snails additionally recorded long-range movement between habitats (Figure 5). Five metrics of movement were calculated per individual.

Movement was extremely variable among individuals; the mean distance from their release point to snails' final positions was 0.96m in ANG and 1.13m in CZB across a mean of 23 and

29 days (excluding the long-range outliers). However, distance from release varied from a minimum and maximum distance of 0.01m to 52.1m and 0.01m to 76.7m in ANG and CZB, respectively (Figure 5).

Differences in movement metrics between ecotypes and release habitats were expected, as an indicator of habitat choice. Ecotypes in a non-native habitat were expected to disperse over greater distances than the native ecotype, and in a more directional (less random) manner (Janson 1983; Erlandsson *et al.* 1998; Cruz *et al.* 2004). For all metrics, it was possible that recaptured snails would appear to show reduced movement compared to non-recaptured snails as a result of the greater difficulty in locating snails that had displaced further from release points during fieldwork.

The along-shore distance metric captured movement parallel to the waterline, corresponding to movement towards or away from other habitats (Figure 6; Supplementary Table 3). Movement of displaced ecotypes towards their native habitat was expected while ecotypes released into their own habitat may move more randomly. In ANG, the along-shore distance moved varied by ecotype ($F=4.5$, $df=2$, $p<0.05$), specifically within the Hybrid release habitat ($F=5.8$, $df=2$, $p<0.001$). Here, the Crab ecotype moved towards the Crab habitat (indicated by negative values) while the Wave ecotype moved towards the Wave habitat (indicated by positive values) and Hybrid ecotype movement was in both directions roughly equally (centred around zero; Figure 6). A similar trend is suggested in the Crab habitat where Hybrid and Wave ecotypes moved in a relatively more positive direction than the Crab ecotype (Figure 6). No significant differences in along-shore movement were present across CZB individuals. Within a few groups, along-shore movement differed with recapture success: non-recaptured snails moved a little more positively in the Crab ecotype in ANG ($F=4.0$, $df=1$, $p<0.05$), while in CZB movement varied contrarily with recapture success between release habitats in the Wave ecotype ($F=7.8$, $df=2$, $p<0.001$) and between ecotypes in the Crab release habitat ($F=6.7$, $df=2$, $p<0.01$).

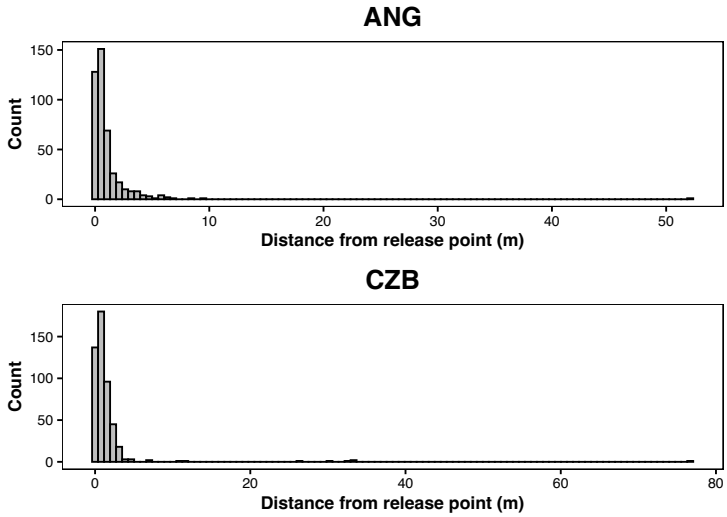


Figure 5. The distribution of straight-line distances between the release point and point of last encounter (recapture of movement) for all snails in each site.

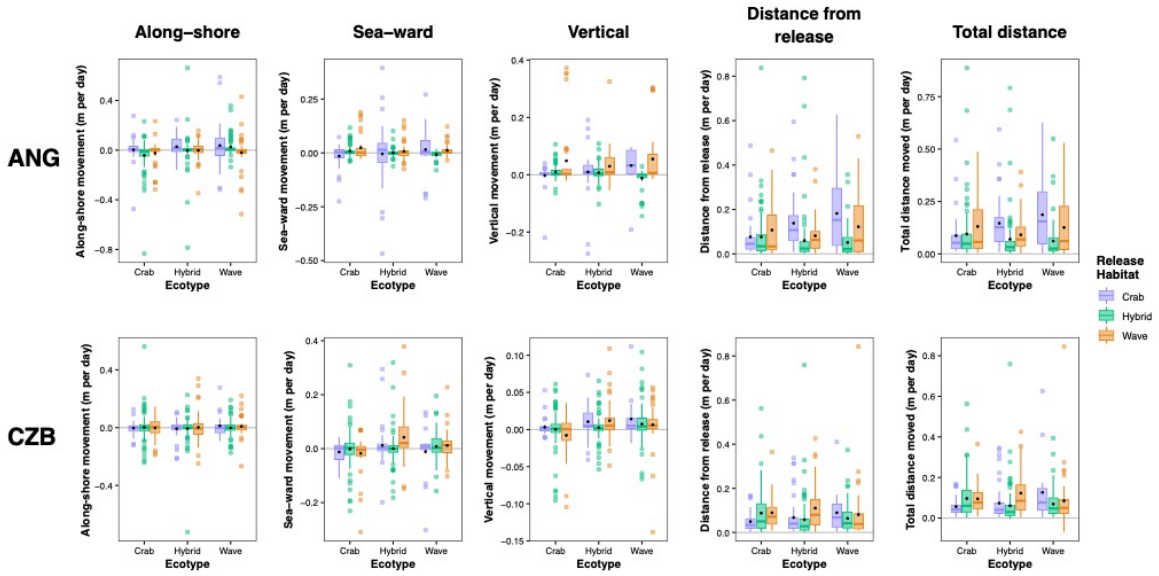


Figure 6. The five movement metrics (along-shore movement; seaward movement; vertical movement; distance from release point; and total distance moved- all standardised by number of days) for all snails, per ecotype and release habitat in each site. Black points indicate mean values for each group.

Movement towards or away from the waterline was captured by the seaward movement, where positive values indicated movement away from the sea and negative towards (Figure 6; Supplementary Table 3). Transplanted individuals have previously been shown to return to the shore height from which they were displaced (Erlandsson *et al*. 1998; Cruz *et al*. 2004); this is expected to be reflected in seaward and vertical movement. In CZB, seaward movement differed between ecotypes ($F=3.8$, $df=2$, $p<0.05$) and this relationship further varied between release habitats ($F=2.6$, $df=4$, $p<0.05$; Figure 6). The ecotype differences were prominent in the Wave release habitat ($F=5.3$, $df=2$, $p<0.01$), where Crab snails moved towards the water line and Wave and Hybrid snails moved away from the water line with increasing distances (Figure 6). In contrast, seaward movement distances were smaller in other release habitats with little difference between ecotypes. Within the Hybrid release habitat at ANG, Crab snails moved away from the waterline while Wave snails moved towards and Hybrid snails moved similarly in both directions ($F=9.2$, $df=2$, $p<0.001$; Figure 6). Crab ecotype individuals moved differently among release habitats ($F=6.4$, $df=2$, $p<0.01$); in their native habitat, movement was largely non-directional, maybe biased toward the sea, whereas individuals moved away from the water's edge by increasing amounts in the Hybrid and Wave habitats. Seaward movement differed according to recapture success in different manners in ecotypes ($F=3.4$, $df=2$, $p<0.05$) and release habitats ($F=3.3$, $df=2$, $p<0.05$). Generally, greater distances were moved in the Crab release habitat than others (Figure 6).

Vertical movement, i.e., up or down boulders/cliff, was also characterised. Multiple differences between ecotypes and release habitats were apparent (Figure 6; Supplementary Table 3). In CZB release habitats, native ecotypes appeared to move less than their non-native counterparts. As with seaward movement, ecotypes moved in different directions ($F=7.0$, $df=2$, $p<0.001$); Crab individuals moved downwards while Hybrid and Wave individuals moved upwards (Figure 6). This was visible in the three habitats, but significant only within the Wave

habitat ($F=5.1$, $df=2$, $p<0.01$). Similar patterns were discernible but insignificant in Crab and Wave habitats in ANG. However, ecotypes moved the opposite directions within the Hybrid habitat ($F=11.0$, $df=2$, $p<0.001$); Wave snails moved downwards while Crab and Hybrid snails moved upwards (Figure 6). In general, all individuals moved smaller distances in the Hybrid habitat than the other two ($F=20.6$, $df=2$, $p<0.001$). Accordingly, the varying relationship between ecotype and release habitat in ANG was also significant ($F=2.8$, $df=4$, $p<0.05$). Snails that were not recaptured tended to move further than recaptured snails in ANG but the difference between groups depended on release habitat ($F=6.4$, $df=2$, $p<0.01$).

The overall distance of travel for each snail was summarised by two non-directional movement measures- distance from release and the total distance moved (Figure 6; Supplementary Table 3). Aside being indicators of distance, similar values of the two metrics for an individual suggested directional movement from the release point while total distance moved being greater than distance from release implied more random movement. As above, expectations for distance and direction of movement were different between snails transplanted to non-native environments and their native counterparts. Patterns of movement between ecotype and release habitats were equivalent across the two metrics so were considered together. In both sites, distance from release (DR) and total distance moved (TD) varied with release habitat (Figure 6) and recapture success. In ANG, snails moved the smallest distances in the Hybrid and largest in the Crab habitat (DR: $F=22.5$, $df=2$, $p<0.001$; TD: $F=21.6$, $df=2$, $p<0.001$) and recaptured snails moved much shorter distances than non-recaptured snails (DR: $F=33.6$, $df=1$, $p<0.001$; TD: $F=33.0$, $df=1$, $p<0.001$). Recaptured snails also moved least in CZB (DR: $F=21.1$, $df=1$, $p<0.001$; TD: $F=15.8$, $df=1$, $p<0.001$) but at this site movement distances were greatest in the Wave habitat and similarly low in Hybrid and Crab habitats (DR: $F=4.8$, $df=2$, $p<0.01$; TD: $F=9.1$, $df=2$, $p<0.001$). The level of movement across release habitats was not consistent between recaptured and non-recaptured snails- the overall pattern between habitats as described above remained true in recaptured snails, whereas movement was greatest in the Crab habitat for snails that were not recaptured (DR: $F=6.1$, $df=2$, $p<0.01$; TD: $F=4.1$, $df=2$, $p<0.05$). In CZB, the native ecotype in each habitat moved a smaller distance from the release point than non-native ecotypes, and each ecotype moved the least distance from release when transplanted into their native habitat ($F=2.7$, $df=4$, $p<0.05$; Figure 6). The same trend is also visible (but non-significant; $F=3.1$, $df=2$, $p=0.05$) in the Crab habitat in ANG.

Altogether, a variety of significant movement trends were detected across the five movement metrics (Figure 6). Along-shore movement differed between the ecotypes in the expected directions, especially in the Hybrid habitat. The predicted pattern of distance moved was also visible in both distance metrics, with a significant ecotype*habitat interaction. Differences in seaward and vertical movement between ecotypes and/or habitats were also detected in multiple tests, but showed differing trends in tests of different groups so needed to be considered separately with respect to the specific habitat conditions in each case as to whether they followed expectation.

Survival and recapture probabilities

Sighting (movement) and recapture information from all snails was included in the RMark analysis of apparent survival and encounter probabilities. Survival probability was predicted to change over time and in the opposite direction between release habitats for each ecotype, with the highest survival of each ecotype being in their native habitat. Hybrid snails were expected to show lower survival in other habitats than Crab or Wave snails in the hybrid zone.

Time was predicted to affect survival probability, with recapture probability also likely to vary by time, ecotype, and release habitat.

An initial exploration of a simple recapture rate (proportion of snails that were recaptured; F_x snails excluded from statistical test) before using RMark showed that the rate differed between ecotypes at both sites (ANG: χ^2=15.5, df=2, p<0.001; CZB: χ^2=8.3, df=2, p<0.05) and that this relationship varied across release habitats in CZB, i.e. the interaction term was highly significant (χ^2=17.7, df=5, p<0.01). Ecotypes often had the highest recapture rate in their native environment but this was not consistent across sites (Figure 2; Supplementary Table 4). Trends were investigated in more depth in RMark since the program had the ability to distinguish survival probability from encounter probability. Results described are based on model sets D1 and D2 unless otherwise stated, as these were the full, most complex model sets that accounted for a variable encounter probability and tested all variables in survival formulae (Tables 2-4; see Supplementary Table 5 for model selection tables of all model sets, A1-D2).

The apparent survival probability (ϕ) of snails was highly affected by ecotype, release habitat and time across all model sets. The most informative model sets were D1 and D2, where a variable encounter probability (*p*) was accounted for while examining factors that affect ϕ. Across time, the daily survival probability mostly remained above 0.9 for all groups but drops down to almost 0.2 did occur (explored in more detail below; Figure 7). In each release habitat in ANG, the native ecotypes showed the highest survival, although the survival probabilities of the Hybrid and Wave ecotypes were almost identical in the Wave habitat (Figures 7-8). In contrast, the Hybrid ecotype in CZB was most likely to survive in the Crab and Hybrid habitats and again equally likely as the Wave ecotype to survive in Wave. Across both sites, ϕ estimates for the Crab and Hybrid ecotypes were highest in the Crab release habitat and lowest in the Wave habitat, but the Wave ecotype showed a different trend (Crab>Wave>Hybrid habitat in ANG and Hybrid>Wave>Crab habitat in CZB; Figures 7-8). Notably, the Wave ecotype experienced the smallest variation in ϕ between habitats of all the ecotypes whereas survival of the Crab ecotype changed to a much greater degree between habitats. Conspicuous reduction in ϕ within a habitat compared to the native ecotype were evident for the Crab ecotype in the Wave habitat and vice versa at both sites (Figures 7-8), highlighting the crossing reaction norms, i.e., the Wave ecotype was more successful than the Crab ecotype in the Wave habitat and the Crab ecotype more successful than Wave in the Crab habitat. The expectation for patterns of hybrid survival was not so clear, but the trend that was detected was certainly not expected: hybrids overall showed the greatest survival across the three habitats (Figure 7). Patterns of survival between ecotypes in the Hybrid habitat were comparable to those in the Wave habitat at both sites; in contrast, patterns of survival of the Hybrid ecotype between release habitats were comparable to those of the Crab ecotype (Figures 7-8).

The apparent survival probability fluctuated across the course of the experiment at both sites (Figure 7). Variations remained between 0.9 and 1.0 at all times aside for one large drop in ϕ (representing a concentration in mortality) that was experienced at the same time within each site for all ecotype/release habitat groups (Figure 7). At ANG, the large drop in ϕ to around 0.35 occurred at day 153, indicating a greatly reduced probability of survival from the previous day sampled- day 91- to day 153. At CZB, ϕ was reduced to around 0.76 for the period of days

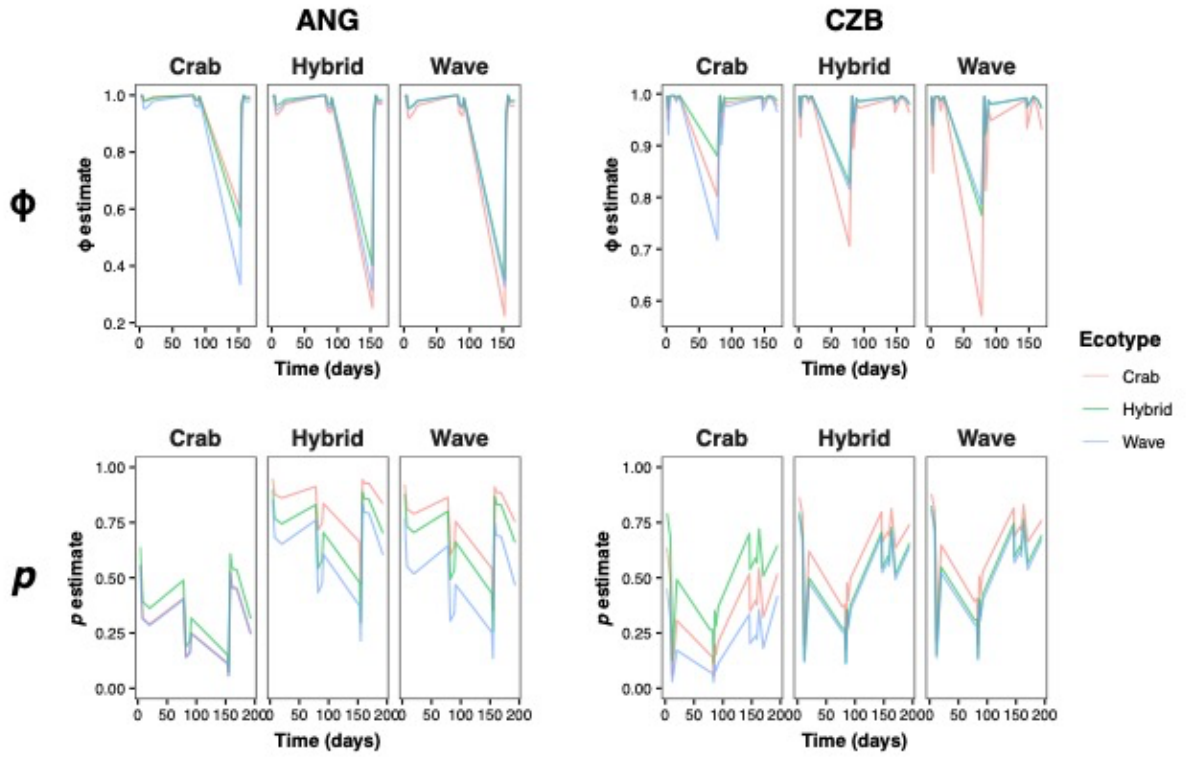


Figure 7. Estimates of apparent survival probability (ϕ) and encounter probability (*p)* over time for each ecotype in each release habitat at each site. Estimates were extracted from the averaged model from RMark model set D1 (Tables 2-4), where all models from the set were ranked by AICc value and averaged, weighted by ΔAICc to the best fitting model.

20 to 78. Although included in some models, snail weight, shell thickness, and wave action were all found to have no discernible impact on survival probability: confidence intervals of predicted estimates were orders of magnitude greater than the predicted effect on survival.

Tests of encounter probability (*p*), both when ϕ models were kept constant (model sets B and D1; Tables 2-4) and when they varied (model sets C and D2; Tables 2-4), confirmed that *p* was affected by ecotype, release habitat and time. Encounter probability varied across a large range- between 0.03 and 0.95- during the experiment (Figure 7). Changes in encounter probability over time were often as large as differences in encounter probability between ecotype and release groups, highlighting the similar importance of all three factors (Figure 7). Interaction of ecotype or release habitat with time was not permitted in the formulae tested so the order of encounter probability between ecotypes in each habitat remained the same over time.

In ANG, encounter probability for each ecotype was considerably lower in the Crab habitat than the other two habitats- matching experience in the field- and highest in the Hybrid habitat aside from the Hybrid ecotype where *p* was the same between Hybrid and Wave habitats (Figures 7-8). The sizable drop in encounter probability in the Crab habitat compared to others was also evident in CZB- again, as expected- for both Crab and Wave ecotypes; *p* was similar between the Hybrid and Wave habitats for all three ecotypes and also the Crab habitat for the Hybrid ecotype (Figures 7-8). As with ϕ, patterns of encounter probability between ecotypes

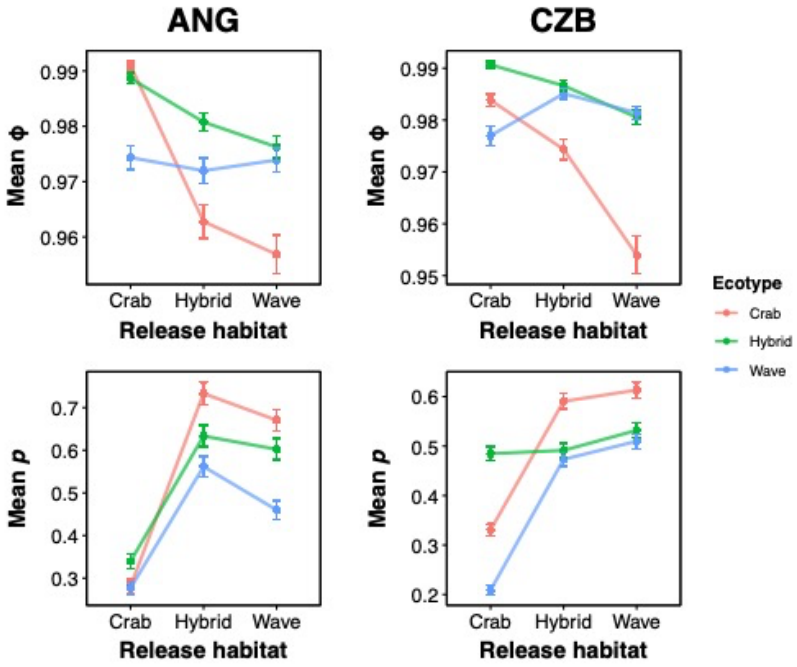


Figure 8. Mean and standard error of estimates of apparent survival probability (ϕ) and encounter probability (*p)* over time (excluding the single large drop at each site) for each ecotype in each release habitat in each site. Each line connects the mean values in each habitat for an ecotype.

in the Hybrid habitat were the same as those in the Wave habitat at both sites. As previously described, although other environmental variables were probably intermediate, the hybrid zones had a rock substrate as in the Wave habitats, so this pattern was not unexpected. The Crab ecotype was most likely to be encountered, followed by Hybrid and then Wave ecotypes in ANG and both equally in CZB. The Hybrid ecotype was the most likely ecotype to be encountered in the Crab habitat both in ANG and CZB, followed by both other ecotypes equally in ANG, or Crab than Wave in CZB.

Overall, survival probability followed the predicted trends for Crab and Wave ecotypes across their native and non-native habitats, even when controlling for the anticipated variation in encounter probability between ecotypes, habitats, and time. Patterns in survival probability of the hybrid ecotype across habitats in relation to the two other ecotypes was a surprising result; similarly, some aspects of the fluctuation in survival probability over time were unexpected.

4.5 Discussion

An extensive reciprocal transplant mark-recapture experiment was carried out on two Swedish populations of *Littorina saxatilis* to test for evidence of divergent selection and habitat choice between locally adapted ecotypes, and investigate the role of selected chromosomal inversions, including those associated with sex determination. The experiment showed differential survival and movement of ecotypes across habitats in addition to significant differences in sex-linked inversion frequencies across these groups.

A surprisingly low number of F_x snails were recaptured- only 15 in total across both sites- potentially related to their very small size. They were excluded from analyses due to this very low number. The moderate recapture rates of 26 and 32% in the experiment included recovery of snails of all factorial ecotype, release habitat and sex combinations. However, sample sizes within some groups were limited (Table 5) which likely affected the reliability of estimates and reduced the power of analyses that could include only recaptured snails. Survival and movement analyses were not affected by this and provided new estimates for survival and dispersal. Excluding the single large drop in each site, daily apparent survival probabilities across the experimental period were a mean of 0.98 in both sites but fluctuated between approximately 0.8 and 1. Snails (excluding the long-range outliers) moved a mean of 0.96m and 1.13m from their release points in a mean of 23 and 29 days in ANG and CZB, respectively, with a mean daily dispersal of 0.09m and 0.07m. Individuals transplanted into their native habitat were recaptured as far as 9.3m from their point of release, much greater than previous estimates of dispersal- 1-2m per month and 1-4m per three months (Janson 1983; Erlandsson *et al*. 1998). Multiple individuals were recorded in habitats other than where they were released, however; this included distances as high as 75m from their release point and highlights that long-distance dispersal may be relatively common. These individuals were likely dislodged from the shore and washed across to different habitats by the sea. Other Littorinids have previously been shown to survive this form of transport (Miller *et al*. 2007) and it must occur in *L. saxatilis* because of the colonisation of skerries observed by Johannesson and Johannesson (1995).

Dispersal and habitat choice

While habitat choice has been much studied from an ecological perspective (Mayor *et al.* 2009), evidence is emerging that habitat choice can play a role in adaptative divergence and speciation- this field remains relatively young and empirical studies that test this are needed (Porter and Akcali 2020). Therefore, movement was broken down into multiple components to test for evidence of dispersal and habitat choice through differences between ecotypes and release habitat along with recapture success. Direction of movement was characterised by along-shore, seaward and vertical movement metrics while distance from release and total distance moved described magnitude of movement (i.e., distance). All five metrics gave evidence of some form of movement differences between ecotypes and/or release habitats (Figure 6).

Along-shore movement differed between ecotypes in ANG, in particular in the hybrid zone. Individuals of the Crab ecotype released into the hybrid zone moved back along the shore towards the Crab habitat and Wave individuals moved in the opposite direction towards the Wave habitat, while movement of hybrid snails was not biased in one direction. Additionally, in the Crab habitat, hybrid and Wave snails appeared to move more positively, i.e. in the direction of their native habitats, than Crab individuals. This represents non-random directional movement of ecotypes towards their habitat of origin, as has previously been shown (Erlandsson *et al*. 1998; Cruz *et al*. 2004). It would suggest that individuals are choosing to move towards habitat that their phenotype is adapted for, and where their fitness will be higher, displaying matching habitat choice (Edelaar *et al*. 2008). Studies have concluded that habitat choice is occurring in species such as toads (MacCallum *et al.* 1998), *Drosophila* (Taylor 1986), roe deer (Gaudry *et al*. 2018) and killifish (Angeletti *et al.* 2017), and matching habitat choice has been shown experimentally in other species, such as grasshoppers (Camacho *et al.* 2020). Matching habitat choice is thought to be important in multiple aspects of adaptation

and speciation (Edelaar *et al.* 2008). Furthermore, the distance that ecotypes moved from their release position varied between habitats in ANG. Within each release habitat, the native ecotype moved the least distance compared to other ecotypes and each ecotype moved the least distance when in their native habitat compared to other habitats, corroborating the findings of Janson (1983) and Cruz *et al*. (2004). An increase in movement when away from their optimal environment supports that individuals disperse further to find areas where they are more fit and move less when in an optimal environment so that they remain there (Webster *et al.* 2012). Once more this is indicative of habitat choice behaviour in all three ecotypes. Spatial distribution of individuals as a result of habitat choice affects mating opportunities; differential dispersal of ecotypes to different areas will decrease the likelihood of hybridisation between ecotypes, i.e. promote assortative mating. This forms an important element of reproductive isolation and therefore contributes to the maintenance of the hybrid zone and distinct ecotypes. MacCallum *et al.* (1998) also concluded that habitat preference helps to maintain the hybrid zone and reduce gene flow in *Bombina bombina* toads. Transplanted stickleback from different environments were shown to disperse back to their native habitats, with this habitat preference increasing the extent of adaptive divergence between the populations (Bolnick *et al.* 2009). The effect of habitat choice in reducing gene flow and contributing to adaptive divergence has been shown by models (Bolnick and Otto 2013; Berner and Thibert-Plante 2015; Kyogogu and Yamaguchi 2023), but the specific mechanisms by which this occurs must be tested empirically (Porter and Akcali 2020). Species such as *L. saxatilis* will be important for this.

Ecotypes also displayed differences in their vertical and seaward movement, which may reflect a smaller scale of habitat choice within the main release habitats. Habitat preference may be scale-dependent, and different habitat components may be selected at each scale (Mayor *et al.* 2009). In the Wave habitat in CZB, arguably the place where there is greatest variation in conditions in these dimensions, the Crab ecotype moved towards the waterline and the Hybrid and Wave ecotypes away. In parallel, the Crab ecotype moved downwards while Hybrid and Wave ecotypes moved upwards across the three habitats in CZB, with the same pattern visible but non-significant in ANG Crab and Wave habitats. Movement upward and away from the water line by Hybrid and Wave ecotypes may be an indication of an attempt to escape crab predation, especially in the Crab habitat. Downward movement from the Crab ecotype may be to shelter from wave action. The fact that ecotype-specific vertical and seaward movement is consistent across habitats and sites certainly supports that it is a form of habitat choice in response to one or more selection pressures that differentially affect the ecotypes. It is comparable to the demonstration in Spain that snails return to their native shore level after being displaced (Johannesson *et al*. 1995; Cruz *et al*. 2004). Other snails- *L. angulifera* and *Nerita senegalensis*- also show homing behaviour up and down shore after displacement (Antwi and Ameyaw-Akumfi 1987). Habitat choice across heterogenous but contiguous microhabitats has previously been shown in killifish (Angeletti *et al*. 2017). Ecotypes showed an opposite pattern of both vertical and seaward movement in the hybrid zone in ANG, wherein only the Wave ecotype moved downwards towards the water. The rock face in the hybrid zone here is much more vertical, with more crevices in the rock at low levels and crabs less likely to be present; it is likely that these site-specific habitat features and consequent spatial differences in selection pressures led to the different movement pattern of the Wave ecotype here. There is now also increasing evidence for heterogeneity with shore height in the Wave ecotype but not Crab; therefore the collection and grouping of Wave

individuals collected at different shore heights may be a source of variation in behaviour in this study that should be controlled for in future studies.

Survival and divergent selection

Estimating survival rates through mark-recapture experiments has long been a useful tool for testing ecological and evolutionary problems (Lebrenton *et al.* 1992, 1993). More recently, the importance of selection against immigrants in contrasting environments (through reduced survival) as a key contributor to reproductive isolation has been argued (Nosil *et al.* 2005). This can be tested with such experiments, to provide direct evidence for the role of divergent selection and immigrant inviability. Recapture rates- the proportion of released snails that were recaptured- suggested that ecotypes were experiencing differential survival in differing habitats, with lowered survival away from their 'native' habitat (Figure 2). This metric, however, conflates survival with the probability of recapture. Mark, specifically designed for mark-recapture data, is able to distinguish between survival and encounter rates and therefore allows robust analysis of survival probabilities while accounting for variation in encounter rates. Indeed, while there was evidence for the variation of encounter probability with ecotype, release habitat and time, changes in apparent survival probability remained when including these factors.

Survival analysis in Mark gave clear evidence for differences in mortality between ecotypes and release habitats and their interaction (Figures 7-8). Ecotypes experienced reduced survival in the other habitats compared to their own; similarly, within each habitat, the native ecotype was the best surviving. Differences in survival probability were especially apparent with Crab and Wave ecotypes in each other's habitats. These 'crossing reaction norms' match what is expected when locally adapted ecotypes are moved into contrasting habitats (Kawecki and Ebert 2004; Savolainen *et al*. 2013) and provides evidence for the divergent selection acting on *L. saxatilis* to produce and maintain the ecotypes. Reciprocal transplant experiments have given evidence for divergent selection through reduced survival across environments in a number of other species, such as ground finches (Sulloway and Kleindorfer 2013), invasive trout (Westley *et al*. 2013), a desert shrub (DiVittorio *et al.* 2020), great tits (Senar *et al.* 2014), and *Ipomopsis* plants (Campbell 2004). Selection must be strong enough that these differences became apparent across the relatively short time span of this experiment. Daily survival probabilities have been estimated in this study as survival is likely to change on fine timescales; as such, average differences between groups may appear small. However, when considering the cumulative effect of these probabilities over time, it is clear that selection is strong. For example, in the Crab habitat in ANG, the mean apparent daily survival probability over the experiment of the Wave ecotype is 0.974 in comparison to 0.991 for the Crab ecotype. If these rates did not change over three months (an estimated likely average lifespan for *L. saxatilis*), this would equate to a lifetime survival probability of 0.443 for the Crab ecotype but only 0.093 for the Wave ecotype- almost 5x lower. The reduction in survival (in terms of the ratio between Crab and Wave ecotypes) is still more apparent in the Wave habitat of CZB, where the lifetime mean apparent survival probability would drop from 0.178 for the Wave ecotype to 0.014 for the Crab ecotype- almost 13x lower. Strong divergent natural selection was also reported in *Encelia* desert shrubs, with relative fitness differences of over ten times in some reciprocal transplant combinations (DiVittorio *et al.* 2020).

Asymmetric reductions in fitness of locally adapted types between environments are common (e.g. Westley *et al.* 2013; Senar *et al.* 2014; Hanson *et al.* 2016), and highlight the interaction

of genotype and environment in effects on survival. Across habitats, the Wave ecotype experienced noticeably smaller differences in survival than the Crab ecotype. In ANG, the mean daily survival of Wave snails differed by just 0.002 between habitats in contrast to 0.034 for Crab snails. This implies that the Wave phenotype may be more generally advantageous across habitats on the shore whereas the Crab phenotype is beneficial in the Crab habitat only. Along with the generally higher survival in the Crab habitat, this in turn suggests that the selection imposed by crab predation is not as strong as that of wave action. At least, the Wave ecotype is better able to withstand crab predation than the Crab ecotype wave action. This is rather surprising as the experiment took place over summer and so few storms but plenty of crabs, i.e., stronger selection from crab predation than wave action, was expected. It is possible that selection is different between adult and juvenile snails: selection imposed by crab predation may be focussed on juveniles as they are often eaten by small crabs. Since it is likely that only adult snails were released in this experiment (small snails <3mm were excluded as they could not be labelled), it may be the case then that the survival estimates in this experiment only apply to snails that have outgrown predation by young crabs.

Survival of Crab and Wave ecotypes is also differentially impacted in the hybrid zone ('Hybrid habitat'). In both ANG and CZB, the order between ecotypes is similar to that in the Wave habitat- the Crab ecotype has the lowest survival probability, while Hybrid survival is higher than Wave (ANG) or the two are similar (CZB). This would therefore support that the hybrid zone environment is overall more similar to the Wave habitat than Crab, and so wave action is a more dominant selection pressure in this area than crab predation. Selection within the hybrid zone was also found to be more similar to one of the divergent sites than the other in *Ipomopsis* plants (Campbell *et al.* 2023).

The survival characteristics of hybrid snails (here referred to as the 'Hybrid ecotype') across habitats is informative about selection across the shore. Somewhat contrary to expectation, hybrid snails showed the highest probability of survival in the Crab habitat rather than the hybrid zone, and the lowest in the Wave habitat. In this sense, they matched the performance of the Crab ecotype across environments but the drop in survival from one habitat to the next was only small, unlike Crab. Most surprisingly, hybrid snails maintained the highest mean probability of survival across all three habitats (mean daily survival for Crab: Hybrid: Wave ecotypes in ANG- 0.970: 0.982: 0.973, and CZB- 0.971: 0.986: 0.981), and the only individual habitat where they did not have the highest survival was Crab habitat in ANG. This behaviour is unexpected for multiple reasons. For one, as discussed above, the survival of ecotypes in the hybrid zone would imply that the hybrid zone environment is more similar to the Wave than Crab habitat, and that hybrid snails would therefore show better survival in the Wave habitat than Crab. Yet results show the opposite. Perhaps most pertinently, the generalist nature of hybrid snails suggested by the maintenance of high survival across environments is in conflict with what is expected with divergent selection across a hybrid zone. Theory dictates that when divergently selected populations interbreed in a hybrid zone, hybrids are maladapted to the two divergent environments and as such do not spread away from the hybrid zone, maintaining the divergence of populations. However, the high survival of hybrid snails in this experiment would suggest that the hybrid phenotype is optimal across the shore and should spread. In reality, this has not occurred; hybrids are not found distant from the hybrid zone and the Crab and Wave ecotypes remain distinct. It is not clear why this discrepancy has occurred. It is possible, since the experiment spanned only seven months from spring to autumn, that more intense selection against hybrids occurs over winter and that long-term survival of hybrids is

lower than that of the pure ecotypes. As discussed above, survival estimates in this experiment likely only represent adult survival as juveniles were excluded; therefore, decreased fitness of hybrids through low juvenile survival would not be detectable in this study. Other reciprocal transplant studies have also found that hybrids perform as well as the parent types in the divergent environments in terms of survival; this includes a wildflower (Kimball *et al.* 2008), a desert shrub (DiVittorio *et al.* 2020), and sticklebacks (Hanson *et al.* 2016). Fitness of hybrids of *Ipomopsis* plant species from a hybrid zone was reduced when transplanted to the other environments for one genotype while hybrids of the other genotype retained high fitness, although reproduction rather than survival was tested (Campbell 2004). Good performance of hybrids across environments can indicate a lack of endogenous isolation, suggesting exogenous factors are more important in affecting hybrid fitness.

In support of this, survival analyses confirmed the variation in survival probability over the duration of the experiment. Patterns of survival over time were inconsistent between ANG and CZB, highlighting site-specificity in the changing strength of selection over time. However, no clear trends were visible in the fluctuation of survival across time. Crab predation is expected to be highest during the summer months due to increased abundance of predator species whilst wave action is stronger when storms are more frequent during winter. This would give the prediction of decreased survival in the Crab habitat during summer and Wave habitat during winter in addition to greater differences in survival between ecotypes in these habitats at those times. The measure of wave action over time (summarised from weather data from the Swedish Meteorological Institute) was shown to have some impact on survival probability- by inclusion in important models in the model selection tables- but the effect was extremely small and negligible in comparison to that of other factors. The lack of pattern over time in this study may be due to an insufficient timespan for the experiment- it did not include winter. Selection pressures may also fluctuate on a finer time scale than simply seasonally, meaning effects on survival cannot be recognised in this data. Averaging wave data over the relatively long gaps between study periods may have also hidden the effect of a few stormy days; analysing only maximums of wave parameters rather than also means may reveal a trend. Inclusion of specific measurements of selection pressures alongside the experiment, such as abundance of crabs and wave data specific to the experimental sites, are likely to yield more useful insight. Both sites show one large drop in survival, each for a different time period. They correspond to the first break between fieldwork periods (May to July) in CZB and the second (July to September) in ANG. Decreased survival in a different one of the two breaks at each site reduces the probability of it being simply an artefact of the gap in fieldwork. This would imply heightened selection at these times, but it is not clear why this would be the case. The two sites are not identical, however, and vary in a number of environmental factors which may have resulted in this fluctuation in selection. For example, they are differently oriented, with CZB generally more sheltered than ANG from wind and waves except for the far end of the Wave habitat; the hybrid zone is more vertical in ANG than CZB; and the wave area in ANG is predominantly low shore but wide and with a range of height in CZB. These variations give plenty of opportunity to be differently impacted by selection.

Changes in the phenotype of ecotypes between release habitats and according to recapture success could also provide evidence for divergent selection, although recapture success does conflate survival with recapture probability. Weight and thickness records confirmed phenotypic differences between ecotypes as expected (Figure 3). Little evidence was present for changes between release habitats, however. In the Crab habitat, recaptured snails were

heavier than non-recaptured snails. Larger snails are more resistant to crab predation, so this supports the decreased survival of smaller snails from predation in this habitat, although may also be influenced through greater conspicuousness and so increased recapture of larger snails. This was where the largest survival difference was found, so may be the reason for detecting a phenotypic effect here. Despite a lack of evidence for selection from differences between groups, the survival analysis in Rmark did include both weight and thickness in models and showed an effect, albeit very low, on survival- these phenotypes do impact survival above the variation already included in ecotype variation.

Inversions, sex, and divergent selection

Whilst it is known that inversions can vary across environments and be associated with adaptive traits, directly measuring selection on inversions is very challenging and has been done very few times (Berdan *et al.* 2023; Nosil *et al.* 2023), in seaweed flies (Butlin *et al.* 1982; Mérot *et al.* 2020), stick insects (Nosil *et al.* 2023), *Drosophila* (Dobzhansky 1947), butterflies (Chouteau *et al.* 2017) monkeyflowers (Lowry and Willis 2010) and mosquitos (Ayala *et al.* 2013). This instead often inferred from patterns and trends and how they match theoretical predictions, such as clines and the association of inversion genotypes with environment (Berdan *et al.* 2023). Attempts to experimentally test selection on inversions in lab or wild populations are therefore valuable. The inversions genotyped in this experiment were selected due to their hypothesised importance in local adaptation in these populations and, for those on LG12, in sex determination. Inversion arrangements are known to vary in frequency between ecotypes. As such, it was expected that individuals with the inversion arrangements advantageous in a certain habitat would have greater survival and so show increased frequencies and vice versa in the other habitat. Only recaptured individuals could be genotyped. Frequency differences of inversion arrangements between ecotypes remained prominent for all of the inversions (Figure 4a-b). Differences between release habitats, and an association between ecotype and habitat, were not detectable for inversions on LG6, LG14 or LG17 (Figure 4a). It was not possible, as a result, to look any further at the role of these inversions in local adaptation. Only the Wave ecotype in ANG showed a decrease in the frequency of the LGC17.1 arrangement outside the Wave habitat, suggesting that it was selected against in the Hybrid and Crab habitats. This difference between habitats is consistent with the frequency difference observed between ecotypes, so supports the presence of a locus or loci important for local adaptation in this inversion. Similar changes in frequency are somewhat visible in LGC14.1 but are not significant; it is possible that selection on the inversions is low so not enough time had passed for changing frequencies across habitats to become obvious.

The three inversions putatively associated with sex determination that could be genotyped (LGC12.2, LGC12.3 and LGC12.4) all retained a strong association of arrangement frequencies with sex, and an association of this with ecotype (Figure 4b). The Crab ecotype showed larger frequency differences between the sexes than the other ecotypes across habitats, sites, and inversions. For LGC12.4, these effects were true only in ANG. The study that found no evidence for LGC12.3 in CZB similarly saw a lack of sex-association of LGC12.4 (chapter three), likely as a result of increased opportunity for recombination between LGC12.2 and LGC12.4. The replication of this result in this experiment supports that the genotyping of LGC12.3 in CZB here may be unreliable and should taken with caution. We cannot conclude that LGC12.3 is absent- it may be present but rare, leaving little overall restriction of

recombination. However, it is not possible to distinguish these options in this study and as such, LGC12.3 in CZB is not discussed any further.

In contrast to its lack of sex-association, LGC12.4 was the only inversion to show overall frequency differences across release habitats. Differences were visible only in CZB and included an association of frequency differences with both release habitat and sex. Arrangement frequency (of the arrangement more frequent in Crab overall) generally decreased from Crab to Wave in both sexes although males were variable, and frequencies were rarely intermediate in the hybrid zone. The difference in frequency between males and females was lower than for the other sex-linked inversions. Further, both LGC12.4 in ANG and LGC12.2 in CZB showed an interactive effect of ecotype, release habitat, and sex on arrangement frequencies, although patterns were unclear. Ecotype-release habitat interactions are expected if arrangement frequencies in an ecotype differ between habitats according to selection on the locally adaptive loci that they capture. (Eventually, this may result in only release habitat effects on frequency if selection was strong enough for long enough on transplanted ecotypes for their arrangement frequency to match that of the native ecotypes.) The presence of these interactions therefore implies variation in fitness of individuals as a result of divergent selection on inversion genotypes across environments (Berdan *et al.* 2023). Nosil *et al.* (2023) demonstrated differences in survival of different inversion genotypes in different environments (host plants) in stick insects using a lab experiment, and the role of multiple evolutionary processes in the maintenance of the inversion. In that case, the complex interplay of the processes was key to the inversion rather than confounding effects to be untangled. The same may be true here, with additional sex-specific selection adding complexity and impacting the LG12 inversions. Since these inversions are located on a chromosome with an SD locus, and frequency difference patterns also vary with sex, it may support the prediction that sex-specific selection is acting differently across the habitats and may involve sexually antagonistic loci captured in the inversions. Svennson *et al.* (2018) emphasise the value of including sex differences in reciprocal transplant experiments that aim to decipher local adaptation. The fact that the only inversion frequency differences detectable from this experiment are for inversions on a chromosome implicated in sex determination suggests that divergent selection is acting more strongly upon (loci in) these inversions than on the other chromosomes and highlights the importance of sex determination and sex chromosomes in local adaptation.

Conclusion

In concert, this experiment has provided evidence of both divergent selection and habitat choice in ecotypes of *Littorina saxatilis* across environments. Transplanted ecotypes have shown habitat-specific movement and survival probabilities that change in line with the expected spatial distribution of selection pressures, supporting the local adaptation of ecotypes. The divergent selection and habitat choice documented here both promote reproductive isolation, the maintenance of the hybrid zone, and differentiation of the ecotypes. Moreover, arrangement frequency changes of sex-associated inversions due to divergent selection have been demonstrated. The recently discovered sex-linked inversions and chromosome they are on in *L. saxatilis* clearly are important in local adaptation in addition to sex determination. Reciprocal transplant mark-recapture experiments have great utility in the study of the role of divergent selection and habitat choice in local adaptation. Larger scale, both in timescale and number of individuals, replications of this experiment in the future that

include genotyping of all released individuals would offer the opportunity to study the genetic basis of these processes in much more detail.

4.6 References

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CHAPTER FIVE
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General discussion

5.1 Research summary

This thesis has examined multiple aspects of sex determination and adaptive divergence, the role of inversions, the selective pressures driving these processes, and interrelationship among them. Considerable progress has been made in recent years in advancing our understanding of these issues, chiefly in conjunction with the rapid advancement of genomic techniques. However, the diversity of mechanisms and drivers alongside the potential for boundless variation in complex interactions across populations and species has precluded a unified theoretical comprehension of these evolutionary processes. As highlighted in chapter one, a varied set of model systems in a range of taxa is required in order to construct and test theories that incorporate and explain the diversity that exists within and between species.

I used a range of genetic data and techniques, combined with an experimental approach, in order to address these issues and investigate a prospective model system in Swedish populations of the intertidal snail *Littorina saxatilis*. I began by identifying and characterising a genetic sex determination system in operation in one population and clarified the structure of the linkage group involved through detection of sex-linked inversions (Hearn *et al*. 2022). I examined their varying influence across a transect between ecotypes and habitats through cline and association analyses of the relationship between inversions and sex. By introducing other populations with replicate hybrid zones, I broadened the study to investigate population and habitat variation in sex determination and sex-linked inversions and began to elucidate the selective pressures that underly the observed systems. Through this, I demonstrated differences in genotype-sex associations in SNPs and inversions across ecotypes and populations, thus highlighting variation in the influence of this sex determining locus and its incomplete association with inversions. This included ecotype- and sex-specific associations on a further linkage group along with another putative inversion, which supported a multigenic sex determination system with different relative importance of loci across environments. Employment of parallel reciprocal transplant experiments across two populations enabled testing of sex-biased divergent selection in relation to sex-linked inversions, in addition to the examination of factors involved in local adaptation and reproductive isolation in this system, primarily habitat choice and divergent selection. Reduced survival of ecotypes in their non-native habitat compared to the native ecotype and to their home environment demonstrated divergent selection and local adaptation of ecotypes. Habitat choice was also shown by ecotypes in the form of differences in direction and distance of movement that varied across environments. In combination, all of the above provided insight into the mechanisms and drivers of sex determination and adaptive divergence, the relative importance of potential selection pressures, and their potential interplay, between environments, ecotypes, and populations in *Littorina saxatilis*.

In this chapter, I begin by highlighting and discussing key outcomes of my research. I review how they address questions in the field identified in chapter one and synthesise the outcomes to discuss how the work offers broader insights into the relationship between sex

determination, local adaptation, and reproductive isolation across diverging populations. I finish by highlighting future directions and perspectives for research into these areas, including the opportunities that this species can offer.

5.2 Key findings from *Littorina saxatilis* and the insights they offer

First evidence for female-heterogametic sex determination and sex-linked inversions in Littorina saxatilis

The study of the evolution of sex determination and sex chromosomes has suffered from a number of challenges; it is difficult to disentangle causes and consequences for a number of factors, including the role of sexual antagonism and mechanisms of recombination suppression (Charlesworth *et al*. 2005; Ironside 2010; Charlesworth 2017; Ponnikas *et al*. 2018); and the ever-increasing diversity of systems that have been described mean generalisation across populations and species is not possible (Bachtrog *et al*. 2014; Furman *et al*. 2020). As previously discussed, this has left a poor understanding of the drivers and mechanisms for these processes and a need for studies on many species, especially with young or homomorphic sex chromosomes and a labile genomic basis for sex (Beukeboom and Perrin 2014; Palmer *et al*. 2019; Furman *et al*. 2020).

As such, I studied sex determination in populations and ecotypes of *Littorina saxatilis*. The species is described as possessing genetic sex determination (Fretter and Graham 1962) but karyotypic study did not find any strongly heteromorphic sex chromosomes in this or eight other periwinkle species from the same family (García-Souto *et al*. 2018). Therefore, through analysis of SNP genotypes and sex-association, I searched for a signal of undifferentiated, homomorphic sex chromosomes (Palmer *et al*. 2019). As the first key finding of the thesis, I provide the first evidence for a female-heterogametic (ZW) sex-determining system in this species, first identified on one island (chapter two, Hearn *et al*. 2022) but later evidenced on three further proximate islands (chapter three). The Littorinidae family includes over 200 species, the chromosomes of only around 10% of which have been described cytogenetically and of these, no sex chromosomes were shown (García-Souto *et al*. 2018); therefore, the demonstration of a ZW system in this thesis provides not only the first evidence for a sex-determination (SD) system in *L. saxatilis* but also in the Littorinidae family. In fact, relatively little is known about sex determination and sex chromosomes for most caenogastropods, although it is suggested that they are evolutionarily flexible in this group and XO, XY, XY_1Y_2 and ZW systems have all been reported for a few dioecious species (Thiriot-Quiévreux 2003; Collin 2018). Thus the *L. saxatilis* ZW system is an important addition and highlights the utility of genomic techniques to identify homomorphic sex determination systems across gastropods, especially those where karyotypic study did not yield results.

The genetic signal of the SD system mapped to linkage group 12 (LG12); the coincidence of this with the location of the strong QTL for sex (Koch *et al*. 2021) provides good evidence for an SD locus on LG12. Two previous putative inversions had been identified on this linkage group (Faria *et al*. 2019), but through a sex-specific inversion detection approach, I provide evidence for two further inversions in one population: LGC12.2 and LGC12.3 - both associated with sex - that cover the remaining part of LG12 (chapter two (Hearn *et al*. 2022)). In conjunction with linkage mapping and calculation of inversion-sex associations, I show that

the SD system is associated with a complex-structured linkage group spanned by inversions with varying levels of sex-association and corresponding sex-specific patterns of recombination, reminiscent of an undifferentiated homomorphic sex chromosome pair with possible young strata of reduced recombination (chapter two, Hearn *et al*. 2022).

Differences in sex determination and sex-linked inversions across ecotypes and populations: multigenic SD or SD switch?

In addition to diversity among species, variation in SD within species - particularly between closely-located populations across divergent environments - is expected to be vital to understanding the dynamism of SD and its drivers and mechanisms (Abbott *et al*. 2017; Furman *et al*. 2020). In recent times, studies have started emerging with evidence of SD-system differences between populations and their effect on reproductive isolation, although many of these describe the turnover of systems through sex chromosome-autosome fusions (e.g. pine beetles (Bracewell *et al*. 2017), sorrel (Beaudry *et al*. 2022), or killifish (Berdan *et al*. 2021)) and there are many other possible mechanisms and drivers for SD system differences and switches (Beukeboom and Perrin 2014; Palmer *et al*. 2019). In Taiwanese frogs, translocation of sex-linked markers caused switches between XY and ZW systems between populations (Katsumi *et al*. 2022) and other species also show differences in the heterogametic sex between populations (Feller *et al*. 2021; Miura *et al*. 2022). Numerous populations across islands and the parallel origins of ecotypes within these in *L. saxatilis* on the Swedish west coast provided me with an ideal system with which to test for variation of the SD system I uncovered.

Through chapters two and three, I describe the complex diversity I find in *L. saxatilis* sex determination that encompasses both population- and ecotype-based variation. This includes the role of LG12, but I further uncover the influence of another genomic region - linkage group 5 (LG5) – that is also involved in female-heterogametic SD. I highlight the varying influence of LG12 and LG5 in SD within *L. saxatilis* populations as a key outcome of my research and discuss the implications of this on mechanisms and transitions in SD in this species.

While signals of the LG12 SD system dominated in the Crab habitat in the ANG population, comparison with the same habitat in three other populations revealed variation in inversions and sex-linkage. Notably, one inversion (LGC12.3) was not detected in two of the other populations; this may reflect absence of the inversion or its low frequency in those populations which precluded its detection. The apparent lack of LGC12.3 in these populations gave insight on the role of LGC12.4; its lack of sex-association when LGC12.3 is absent but low to moderate level of association when LGC12.3 is present argues against a specific role for it in SD and instead its LD with the region important in sex due to recombination suppression. Further, it supports the predominant importance of the LGC12.2 region over any other part of LG12 in the SD system.

In focussing on LGC12.2, variation in its strength of sex-association within and between ecotypes and the four populations is apparent through heterozygosity, association, and cline analyses. Most markedly, LGC12.2 has little to no signal of sex influence in the Wave ecotype populations. It also showed greatly reduced sex-association in two Crab populations. Separate detection of sex-linked regions within the Wave ecotype revealed a role for LG5 in female-heterogametic SD, again with an apparently varying influence. Definitive evidence of an

inversion on LG5 was not found, although behaviour matches that of a little-differentiated inversion at a very low frequency. At one site only, an inversion on LG17 (LGC17.1) was associated with a peak in genetic sex-association; a further SD locus could be present in this region or it may be in LD with another SD locus elsewhere. This region was not considered further during these studies. In combination, variation in LG12 and LG5 and their inversions in sex-association within and between ecotypes across populations could be indicative of a few features of the sex determination system(s) and the role of inversions, as discussed below.

Use of genomic capture data restricted the identification of actual SD loci since a large proportion of the genome was not sequenced and SD loci were likely missed; I was therefore reliant on signals of sex-linkage of inversions to infer behaviour of any SD loci. As a result, the variation documented may be due to varying association of SD loci with inversions rather than a varying influence of the loci on sex determination. It is therefore possible that SD loci on LG12 and LG5 play the same roles in sex determination across ecotypes and populations - comprising a multigenic SD system - but are not associated to the same extent with inversions on those linkage groups. This in itself can provide insights into selection acting upon loci in the inversions and is discussed below. In contrast, if the SD loci are perfectly associated with the inversions, the variation would be an indicator that multigenic SD has undergone or is undergoing some kind of switch and different loci have different relative importance between habitats - with LG12 and LG5 loci predominant in Crab and Wave, respectively. Both of these possibilities may be occurring concurrently to differing extents and gene flow across the hybrid zone between ecotypes can further muddy their signals. The sex QTL on LG12 was uncovered in families derived from a Crab x Crab cross and was strong but not perfect (Koch *et al*. 2021); this supports that the LG12 SD does not solely determine sex in this ecotype. Although the nature of the data precludes understanding of the exact SD system (or systems) at play, it is clear that genetic sex determination is complex and changeable in *L. saxatilis* populations even within this small area of Sweden.

Divergent selection, habitat choice, and the role of hybrids in local adaptation of ecotypes

Differences in sex determination in populations across contrasting environments can contribute to differentiation and reproductive isolation, but many other factors can also be important. The divergence of such populations when connected through gene flow is a much-studied area yet in many cases the drivers, mechanisms, and their relationships remain uncertain. Local adaptation, habitat choice, and assortative mating have all shown to be important in the differentiation of *L. saxatilis* ecotypes (Janson 1983; Erlandsson *et al*. 1998; Rolán-Alvarez *et al*. 1999; Cruz *et al*. 2004; Hollander *et al*. 2005; Perini *et al*. 2020). In my examination of the interconnected systems of sex determination and adaptive divergence in *L. saxatilis* across my thesis, I considered the roles of divergent selection, habitat choice, and local adaptation.

Clear clines in LG12 inversion arrangement frequency between the ecotypes (chapters two and three), and strong association of arrangement frequency with ecotype for inversions on LG6, 12, 14 and 17 (chapter four) provide clear support for divergently acting selection on the four inversions and infer the importance of these linkage groups in local adaptation of the ecotypes. Variation in LG12 clines between the populations (chapter three) indicate site-specific divergent selection. Snails of each ecotype recaptured from contrasting habitats in the

reciprocal transplant experiment did not differ in their arrangement frequency for most inversions (LG6, 12, 14, 17), however (chapter four): selection did not act strongly enough upon the inversions to produce an observable effect within the time span of the experiment (7 months). The two exceptions to this were both inversions implicated in sex determination: both LGC12.2 and LGC12.4 did show different frequencies in ecotypes across environments.

Local adaptation and divergent selection were experimentally tested through reciprocal transplant mark-recapture (chapter four). Although similar experiments had been carried out in *L. saxatilis* in the past, it has previously been difficult to disentangle the effects of survival and dispersal and direct estimates of these factors were not calculated (Webster *et al*. 2012). Through a combination of repeated short-term sighting and long-term recapture efforts in combination with analysis with Mark - a dedicated program for mark-recapture data - I was able to test directly for differences in survival probability while controlling for variation in recapture probability. Here, I provide evidence for divergent selection through differential survival of locally adapted ecotypes in contrasting environments. Across both sites, within the Wave habitat the Crab ecotype showed greatly reduced survival compared to the Wave ecotype, and vice versa in the Crab habitat. In the hybrid zone, survival of both ecotypes was generally lower than in their native habitat. Individuals were selected against in habitats different to that which they were adapted to.

Whilst the survival of ecotypes across habitats confirmed the pattern expected, the behaviour of hybrid individuals transplanted to Crab and Wave habitats was a highly surprising finding. It was expected that hybrids would suffer reduced survival compared to the native ecotype in each habitat due to being less well adapted. However, the opposite trend was apparent: hybrid individuals showed as high, or higher, survival in both habitats at both sites as the native ecotypes. Coupled with their greater survival in the hybrid zone than the two ecotypes, hybrid individuals were the best surviving across the entire environmental gradient. The implied generalist nature of hybrid zone individuals is in direct conflict to what theory dictates about the maladaptation of hybrid offspring of divergent populations (Kirkpatrick and Barton 2006) and suggests that the hybrid phenotype should spread across the habitats. This has not happened in reality, but why this irregularity occurred in the experiment is not clear. It may be due to seasonally varying or age-dependent selection on phenotypes which was not prominent in the duration of the experiment.

Dispersal behaviour of transplanted snails was in agreement with that expected and demonstrated habitat choice that supported the action of divergent selection on ecotypes. Most notably, when released into the hybrid zone, both ecotypes moved in the direction of their habitat of origin (i.e., in opposite directions to one another), while hybrids moved non-directionally. The same behaviour was visible in the Crab habitat where the native ecotype did not show directional movement, but the Wave ecotype and hybrids moved along the shore towards their native habitats. Habitat choice was also apparent through distance of movement. Individuals transplanted into non-native habitats dispersed further than in their native habitat, and within each habitat, the native ecotype dispersed the smallest distances. Habitat choice promotes assortative mating and local adaptation (Maynard Smith 1966; Rice 1984; Webster *et al*. 2012; Camacho *et al*. 2020). The demonstration of the separate but concurrent action of both divergent selection and habitat choice in the same experiment provides evidence for their importance in the maintenance of ecotype differentiation in *L. saxatilis.*

The same inversions involved in both sex and ecotype differentiation

Inversion arrangements are frequently evidenced to differ between groups (sexes or populations) (e.g. Lahn and Page 1999; Joron *et al*. 2011; Wang *et al*. 2014; Lee *et al*. 2017; Fuller *et al*. 2018; Natri *et al*. 2019; Shearn *et al*. 2020), are hotspots for the accumulation of loci important in their differentiation (Sun *et al*. 2017; Furman *et al*. 2020), and are thought to be a mechanism to initiate or preserve linkage disequilibrium between such loci (Charlesworth 1991; Kirkpatrick and Barton 2006). The concurrent role of the same inversions for both processes is therefore possible in some instances. In this thesis, I find support that inversions on LG12 that are associated with ecotype divergence are also important in sex determination. Arrangement frequencies of primarily LGC12.2, but also LGC12.3 and LGC12.4, show sex-specific clines between ecotypes (chapters two and three) and are affected by sex, ecotype, and habitat interactions in a reciprocal transplant (LGC12.2 and LGC12.4; chapter four). Furthermore, regions of LG12 are associated with traits that are sexually dimorphic and divergent between the ecotypes (Morales *et al*. 2019; Koch *et al*. 2021; Koch *et al*. 2022), highlighting the joint importance of the LG12 inversions in both processes. Both sex- and ecotype-specific selection must be acting on LG12 and jointly shaping its behaviour across environments; this is discussed below.

While much less prominent than LG12, I show suggestive evidence that another two genomic regions may support sex as well as ecotype differences. In two sites, LG5 showed sex-association in genotypes that was restricted to the Wave ecotype. This linkage group has previously been shown to hold some outlier SNPs relating to Crab-Wave divergence (Morales *et al*. 2019) and is associated with phenotypic traits that are sexually dimorphic and divergent between ecotypes (see below; Koch *et al*. 2021; Koch *et al*. 2022). Together, this supports LG5 as a genomic region involved in sex and ecotype differentiation. Its behaviour also matches that of an inversion of low frequency and differentiation but there was not robust evidence in this study for the presence of an inversion in this location, so LG5 cannot yet provide evidence for the joint role of inversions in both processes. Sex association was also present for LGC17.1 in the Wave ecotype at one site (chapter three). The frequency of LGC17.1 is associated with ecotypes (chapter four and Faria *et al*. (2019)). Whether due to its capture of an SD locus or LD with another region important in SD, the sex association suggests that some level of sex-specific selection is impacting this inversion in addition to its role in ecotype adaptation.

Selection pressures driving sex determination and ecotype divergence and the importance of LG12 and LG5

Sex- and ecotype-specific selection in *L. saxatilis* and its association with genomic regions implicated in SD is a key outcome and is supported through multiple elements of the thesis. This includes the significant effect of the interaction between sex, ecotype and release habitat on the arrangement frequencies of LGC12.2 and LGC12.4 (chapter four), and the differing clines between males and females for LGC12.2-12.4 (chapters two and three). Through the coincidence of genomic regions that are variable between sexes or ecotypes with environmental variation, alongside their association with adaptive or sexually dimorphic traits, one can begin to identify potential selection pressures that are driving these patterns.

Multiple phenotypic components that are divergent between ecotypes - including shell shape parameters, weight, and boldness - are associated with LG12 and some are also sexually

dimorphic (Morales *et al*. 2019; Larsson *et al*. 2020; Koch *et al*. 2021; Koch *et al*. 2022). This supports the suggestion that selection pressures important in sex determination and ecotype adaptation are acting on some of the same traits and specifically supports a role of environment-dependent sex-specific selection. In particular, the shell shape parameter 'height-growth' varies in its extent of sexual dimorphism between ecotypes - it was shown to be dimorphic in the Wave ecotype but not the Crab ecotype in the three sites tested (the same sites as in chapter three) in addition to being divergent between ecotypes (Koch *et al*. 2022). Furthermore, LGC12.2 has a significant effect on variation in the height-growth parameter.

Shore height is also likely to be an important environmental axis in which sex- and ecotype-specific selection are acting. LG12 is already implicated in divergence between the low and high shore (Morales *et al*. 2019), and phenotypic traits that show habitat*shore height effects are often both sexually dimorphic and influenced by LG12 and LG5 (Koch *et al*. 2021; Koch *et al*. 2022). Shore height shows greater variation in the Wave than Crab habitats, and concurrently the Wave ecotype is the more sexually dimorphic. The Wave habitat in CZB showed the greatest drop in survival of the Crab ecotype compared to Wave out of all habitats across both sites in the transplant experiment (chapter four), and this is also probably the habitat with the greatest variation in shore height. Altogether, this is suggestive of the conjoined impact on phenotypic traits of selection based on sex, ecotype, and shore height differentiation and the role of LG12 and LG5 in their genomic basis.

In combination with the role of LG12 in sex determination, it is therefore clear that this linkage group is a key genomic region in the maintenance of ecotype and sex differentiation and that selection on the same phenotypic characteristics are likely to be driving both. The evidence for loci of sexually dimorphic, and so sexually antagonistic, traits in the same region as an SD locus provides another example of the coincidence of these loci as seen across many other species. Popular theory would thus support that the sexually antagonistic loci on LG12 were a driver for the evolution of the sex-linked inversions, although as previously discussed, prior presence of the inversions may have conversely provided an ideal location for the accumulation of the sexually antagonistic loci. At this point, it is not possible to distinguish between the scenarios.

Multiple sexually dimorphic shell shape traits, predominantly aperture size and shape but also height-growth, are influenced by LG5 in addition to LG12 (Koch *et al*. 2021; Koch *et al*. 2022). The aperture parameters are also divergent between ecotypes in the three sites. In one site, as with height-growth, they are sexually dimorphic in the Wave ecotype but not Crab ecotype (Koch *et al*. 2022). Once more, the sexual dimorphism of traits that have shown to be important in ecotype adaptation and the influence on them of a region with an SD locus gives strong support for selection that is jointly sex- and ecotype-specific. The varying strength of selection imposed by wave action and crab predation across ecotype habitats is well-documented and their effect on phenotypes including shell shape widely studied (Boulding and Van Alstyne 1993; Johannesson *et al*. 2010; Boulding *et al*. 2017; Le Pennec *et al*. 2017). The sexual dimorphism of these traits highlights possible sexually antagonistic selection upon them; shell shape may play differing roles in mate choice and/or the physical success of mating within and between ecotypes, as possible drivers of this selection. The persistence of LG12 sex differences into the Wave ecotype in habitat areas where it is more sheltered and thus more 'Crab-like', including the gully in one site, supports the action of sex-specific selection on adaptive traits (chapter three).

Crosses have recently been used to reveal how polygenic sex determination in a cichlid species, *Metriaclima mbenjii,* that includes both ZW and XY loci produces modular sexual polymorphism on more axes of variation than would be possible with a single SD locus (Moore *et al*. 2022). They suggest that gains, losses, or switches in SD loci across populations can facilitate more optimal variation in sexual dimorphism and impact spatial and temporal variation in traits and fitness. This mechanism could explain the diversity in sexual dimorphism and sex determination across environments in *L. saxatilis.*

Labile habitat-based SD associated with adaptive divergence in Littorina saxatilis*: a system for untangling these processes*

The importance of finding model systems with labile sex determination and its variation across environments in order to understand the mechanisms and drivers that underly the process is clear and has been discussed throughout the thesis. As the final key finding, I wish to reiterate the outcome from across the chapters that *L. saxatilis* shows the exact qualities required for future study systems in this area. In addition, the amenability of this species to lab- and field-based experimental approaches and ease of widespread sampling may leave it a much more feasible option than other candidate systems. Through my work on sex determination, habitat-based divergence, and the role of inversions in these populations, I recommend that *L. saxatilis* be utilised in the future to help solve the multiple outstanding questions in the field. The incomplete associations between sex and inversions contribute to providing a powerful system in which to untangle their effects.

5.3 Future perspectives and directions

A range of data and analytical and experimental techniques were utilised throughout the present study to uncover first evidence of many complex evolutionary relationships between sex determination, adaptive divergence, and the role of inversions in Swedish *L. saxatilis*. The outcomes themselves contribute to the collective understanding of such processes, but also offer a standpoint to inform, and upon which to base, further work in *L. saxatilis* and beyond.

An intricate relationship between sex determination and inversions in divergent ecotypes across environmental transitions was uncovered and described. The use of genomic capture data precluded identification of specific SD loci or the separation of their effects from the behaviour of sex-linked inversions. Use of whole genome sequencing (WGS) data from individuals across the changing environments will allow the separation and examination of these effects; thus, WGS approaches will facilitate understanding of SD loci and inversions individually and therefore also their impacts on one another. Recent production of a new, greatly improved genome assembly for *L. saxatilis* after completion of the present study will be of much benefit to this future work.

The high variability in sex determination and genetic patterns within such a small geographical area of the species range highlights a motivation to widen the study across many other populations in multiple countries. A combination of parallelism and unique trends between populations across a much more diverse set of environments will greatly aid in the understanding of the interface between environment- and sex-based selection. Distribution

and behaviour of SD loci and sex-linked inversions across the species range can also help infer the age and order of origin of these genetic features. Furthermore, investigation of sex determination across many areas of intermediate environment where there is no clear typical Crab or Wave habitat may show its decoupling from divergence effects and therefore be a valuable tool in the understanding of sex determination separately from its interrelationship with adaptive divergence. Inclusion of other closely related species- specifically from the *Littorina* genus- and a wider comparison of their sex determination systems will also be a useful tool in these studies. In particular, some signal has already been found recently in a flat periwinkle, *Littorina fabalis,* of a male-heterogametic (XY) sex determination system with divergent Y haplotypes between ecotypes (A. Le Moan, *pers. comm.*). This further highlights the prospective value of *Littorina* studies in elucidating the complicated relationship between sex determination, adaptive divergence, and inversions.

Lab experiments were instrumental to uncovering the mode of sex determination in another snail- the apple snail *Pomacea canaliculata* (Yusa and Kumagai 2018). Through extensive mating trails and analysis of sex ratio, Yusa and Kumagai (2018) were able to distinguish oligogenic (as defined by authors as a small number (3 to 9) of genes) rather than polygenic sex determination in the species. Crosses between closely related species of cichlid fishes were also used to identify different sex determiners in each species in a clade with extremely high rates of SD turnover (Feller *et al*. 2021). It is possible that similar techniques could be utilised in *L. saxatilis* to help understand the varying genomic basis of sex, particularly if sex determination is shown to differ across the species range. Furthermore, the crosses of a species of cichlid with a polygenic SD system helped explain diverse, modular sexual polymorphism (Moore *et al*. 2022); given the variation in sexual dimorphism and sex determination across populations in *L. saxatilis*, similar experiments may be valuable in this system and help to elucidate sources of sex-specific selection. Lab experiments can also be used for mapping of other traits important to reproductive isolation (RI), especially those which are sexually dimorphic, between the ecotypes (e.g. as in stickleback (Kitano *et al*. 2009)). Examining the location of RI loci in relation to SD loci in the genome, i.e. whether they are located on LG12 or LG5, will bring together the mechanisms for local adaptation and divergence and sex determination, to provide a more rounded view of these evolutionary processes in this species.

5.4 Concluding remarks

To summarise, in this thesis I have revealed a female-heterogametic genetic sex determination system in populations of *Littorina saxatilis* that appears to be multigenic and involve multiple linkage groups, providing first evidence for the mechanism of sex determination in this species (Hearn *et al*. 2022). I further show that this SD system is intertwined with the multifaceted divergence of the Crab and Wave ecotypes across hybrid zones and heterogeneous environmental conditions. I find two additional inversions that had not previously been identified in *L. saxatilis* through use of sex-specific detection methods, and characterise the effect of sex and ecotype on the behaviour of these sex-linked inversions (Hearn *et al*. 2022). Through experimental techniques, I demonstrate and quantify habitat-based divergent selection and habitat choice on the ecotypes and hybrids in combination with intricate sex-, ecotype-, and habitat-specific selection on inversions important in sex determination. In combination, my results provide new insight into the interrelatedness of sex

determination, reproductive isolation, and local adaptation and their drivers and mechanisms. They highlight *L. saxatilis* as a valuable new system with which to examine these processes both in combination and separately in order to untangle and understand them.

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APPENDIX A
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Chapter Four supplementary tables

Supplementary Table 1. ANOVA outputs from general linear models of the effect of ecotype, release habitat, and recapture rate on phenotypes (weight, shell thickness, and shell growth) in various datasets. Abbreviations legend at the bottom of the table.

Site	Phenotype	Dataset	Variable	DF	Sum sq	Mean sq	F value	Pr(>F)	Significance
ANG	Weight	Full	recap_yn	1	3.05	3.052	6.3645	0.0119	*
			ecotype	2	353.77	176.884	368.8676	<0.0000000000000002	***
			rel_habitat	2	2.99	1.495	3.1185	0.04495	*
			recap_yn:ecotype	2	0.02	0.008	0.0167	0.98341	
			recap_yn:rel_habitat	2	0.67	0.337	0.7025	0.49575	
			ecotype:rel_habitat	4	3.57	0.893	1.8626	0.11545	
			recap_yn:ecotype:rel_habitat	4	1.91	0.477	0.9951	0.40958	
			Residuals	595	285.32	0.48			
		Crab ecotype	recap_yn	1	0.429	0.42943	1.3761	0.2423	
			rel_habitat	2	0.083	0.04138	0.1326	0.8759	
			recap_yn:rel_habitat	2	0.04	0.01979	0.0634	0.9386	
			Residuals	182	56.795	0.31206			
		Wave ecotype	recap_yn	1	0.755	0.7547	1.3835	0.240838	
			rel_habitat	2	6.458	3.2292	5.9196	0.003157	**
			recap_yn:rel_habitat	2	2.3	1.1501	2.1083	0.124014	
			Residuals	209	114.012	0.5455			
		Hybrid ecotype	recap_yn	1	0.672	0.67192	1.197	0.2752	
			rel_habitat	2	0.022	0.01078	0.0192	0.981	
			recap_yn:rel_habitat	2	0.234	0.1172	0.2088	0.8117	
			Residuals	204	114.515	0.56135			
		Crab release habitat	recap_yn	1	2.969	2.969	5.7037	0.01818	*
			ecotype	2	88.21	44.105	84.7395	<0.0000000000000002	***
			recap_yn:ecotype	2	1.316	0.658	1.2646	0.28536	
			Residuals	149	77.551	0.52			

		Wave release habitat	recap_yn	1	0.137	0.137	0.2152	0.6434	
			ecotype	2	73.599	36.799	57.8156	<2e-16	***
			recap_yn:ecotype	2	0.204	0.102	0.16	0.8523	
			Residuals	157	99.93	0.636			
		Hybrid release habitat	recap_yn	1	0.857	0.857	2.297	0.1307	
			ecotype	2	196.735	98.367	263.6139	<2e-16	***
			recap_yn:ecotype	2	0.396	0.198	0.5311	0.5885	
			Residuals	289	107.84	0.373			
CZB	Weight	Full	recap_yn	1	3.21	3.21	7.5642	0.006119	**
			ecotype	2	227.648	113.824	268.1918	<0.00000000000000022	***
			rel_habitat	2	0.306	0.153	0.3601	0.697753	
			recap_yn:ecotype	2	1.495	0.747	1.7612	0.172644	
			recap_yn:rel_habitat	2	0.086	0.043	0.1019	0.90314	
			ecotype:rel_habitat	4	0.844	0.211	0.4969	0.738014	
			recap_yn:ecotype:rel_habitat	4	1.842	0.46	1.0848	0.363009	
			Residuals	654	277.566	0.424			
		Crab ecotype	recap_yn	1	0.45	0.44964	1.3036	0.2548	
			rel_habitat	2	0.383	0.19162	0.5555	0.5746	
			recap_yn:rel_habitat	2	0.333	0.16661	0.483	0.6176	
			Residuals	218	75.194	0.34493			
		Wave ecotype	recap_yn	1	4.625	4.6247	7.9848	0.005155	**
			rel_habitat	2	0.505	0.2525	0.436	0.64718	
			recap_yn:rel_habitat	2	0.06	0.03	0.0517	0.949614	
			Residuals	218	126.263	0.5792			
		Hybrid ecotype	recap_yn	1	0.478	0.47811	1.3695	0.2432	
			rel_habitat	2	0.242	0.12109	0.3469	0.7073	
			recap_yn:rel_habitat	2	1.534	0.7668	2.1964	0.1137	

			Residuals	218	76.109	0.34912			
		Crab release habitat	recap_yn	1	2.967	2.967	6.8393	0.009759	**
			ecotype	2	47.367	23.6834	54.5932	<0.00000000000000022	***
			recap_yn:ecotype	2	0.551	0.2754	0.6349	0.531281	
			Residuals	162	70.278	0.4338			
		Wave release habitat	recap_yn	1	0.25	0.25	0.5891	0.4439	
			ecotype	2	63.901	31.951	75.4144	<2e-16	***
			recap_yn:ecotype	2	1.172	0.586	1.3828	0.2538	
			Residuals	162	68.634	0.424			
		Hybrid release habitat	recap_yn	1	0.848	0.848	2.0186	0.1563	
			ecotype	2	116.224	58.112	138.3078	<2e-16	***
			recap_yn:ecotype	2	1.755	0.878	2.0887	0.1255	
			Residuals	330	138.654	0.42			
ANG	Thickness	Full	recap_yn	1	0.951	0.9506	4.9299	0.02677	*
			ecotype	2	38.726	19.3628	100.4205	<0.0000000000000002	***
			rel_habitat	2	0.343	0.1716	0.8897	0.41132	
			recap_yn:ecotype	2	0.068	0.0338	0.1754	0.83916	
			recap_yn:rel_habitat	2	0.063	0.0313	0.1624	0.85015	
			ecotype:rel_habitat	4	0.149	0.0373	0.1934	0.94186	
			recap_yn:ecotype:rel_habitat	4	0.67	0.1676	0.8692	0.48209	
		Crab ecotype	recap_yn	1	0.086	0.085593	0.4832	0.4879	
			rel_habitat	2	0.128	0.064195	0.3624	0.6965	
			recap_yn:rel_habitat	2	0.046	0.022766	0.1285	0.8795	
			Residuals	182	32.24	0.177142			
		Wave ecotype	recap_yn	1	0.158	0.15783	0.7751	0.3797	
			rel_habitat	2	0.222	0.11112	0.5457	0.5802	

			recap_yn:rel_habitat	2	0.617	0.30829	1.514	0.2224	
			Residuals	209	42.556	0.20362			
		Hybrid ecotype	recap_yn	1	0.578	0.57842	2.9551	0.08712	.
			rel_habitat	2	0.147	0.07345	0.3753	0.68757	
			recap_yn:rel_habitat	2	0.055	0.02769	0.1415	0.86817	
			Residuals	204	39.93	0.19574			
		Crab release habitat	recap_yn	1	0.635	0.6346	2.5935	0.1094	
			ecotype	2	9.989	4.9943	20.4095	1.47E-08	***
			recap_yn:ecotype	2	0.534	0.267	1.0911	0.3385	
			Residuals	149	36.461	0.2447			
		Wave release habitat	recap_yn	1	0.0608	0.0608	0.3349	0.5636	
			ecotype	2	9.9928	4.9964	27.5422	5.59E-11	***
			recap_yn:ecotype	2	0.0959	0.0479	0.2642	0.7681	
			Residuals	157	28.4812	0.1814			
		Hybrid release habitat	recap_yn	1	0.417	0.4172	2.4218	0.1207	
			ecotype	2	19.104	9.5519	55.4494	<2e-16	***
			recap_yn:ecotype	2	0.1	0.05	0.2901	0.7484	
			Residuals	289	49.784	0.1723			
CZB	Thickness	Full	recap_yn	1	0.323	0.3227	1.6257	0.2028	
			ecotype	2	38.075	19.0374	95.9035	<2e-16	***
			rel_habitat	2	0.037	0.0187	0.0943	0.91	
			recap_yn:ecotype	2	0.442	0.2209	1.1127	0.3293	
			recap_yn:rel_habitat	2	0.124	0.0622	0.3133	0.7312	
			ecotype:rel_habitat	4	0.351	0.0878	0.4423	0.7781	
			recap_yn:ecotype:rel_habitat	4	0.666	0.1664	0.8384	0.501	
			Residuals	654	129.823	0.1985			

		Crab ecotype	recap_yn	1	0.126	0.126392	0.7627	0.3835	
			rel_habitat	2	0.173	0.086529	0.5221	0.594	
			recap_yn:rel_habitat	2	0.101	0.050577	0.3052	0.7373	
			Residuals	218	36.127	0.165721			
		Wave ecotype	recap_yn	1	1.002	1.00214	4.1178	0.04365	*
			rel_habitat	2	0.165	0.08251	0.339	0.71284	
			recap_yn:rel_habitat	2	0.002	0.00095	0.0039	0.99611	
			Residuals	218	53.055	0.24337			
		Hybrid ecotype	recap_yn	1	0.006	0.00637	0.0341	0.8536	
			rel_habitat	2	0.036	0.01808	0.097	0.9076	
			recap_yn:rel_habitat	2	0.672	0.336	1.8023	0.1674	
			Residuals	218	40.641	0.18643			
		Crab release habitat	recap_yn	1	0.243	0.2434	1.1896	0.277	
			ecotype	2	8.944	4.4718	21.8547	3.96E-09	***
			recap_yn:ecotype	2	0.084	0.0418	0.2044	0.8153	
			Residuals	162	33.148	0.2046			
		Wave release habitat	recap_yn	1	0.145	0.1448	0.7317	0.3936	
			ecotype	2	7.632	3.8158	19.2787	3.09E-08	***
			recap_yn:ecotype	2	0.334	0.1669	0.8432	0.4322	
			Residuals	162	32.065	0.1979			
		Hybrid release habitat	recap_yn	1	0.036	0.0358	0.1831	0.669	
			ecotype	2	21.824	10.9122	55.7341	<2e-16	***
			recap_yn:ecotype	2	0.742	0.3708	1.8939	0.1521	
			Residuals	330	64.611	0.1958			
ANG	Growth	Full	ecotype	2	1.633	0.8167	0.4504	0.6389	

			rel_habitat	2	4.939	2.4693	1.3618	0.2619	
			ecotype:rel_habitat	4	9.676	2.4191	1.3341	0.2642	
			Residuals	83	150.503	1.8133			
		Crab ecotype	rel_habitat	2	9.468	4.7339	3.2788	0.05509	.
			Residuals	24	34.651	1.4438			
		Wave ecotype	rel_habitat	2	3.188	1.594	0.7699	0.4733	
			Residuals	26	53.83	2.0704			
		Hybrid ecotype	rel_habitat	2	1.959	0.97957	0.5212	0.5986	
			Residuals	33	62.022	1.87945			
		Crab release habitat	ecotype	2	6.512	3.2560	2.0372	0.1507	
			Residuals	26	41.556	1.5983			
		Wave release habitat	ecotype	2	2.9335	1.4668	1.0203	0.3804	
			Residuals	18	25.8763	1.4376			
		Hybrid releasee habitat	ecotype	2	3.076	1.5382	0.7222	0.4921	
			Residuals	39	83.07	2.13			
CZB	Growth	Full	ecotype	2	2.657	1.32837	1.1976	0.3053	
			rel_habitat	2	0.593	0.29648	0.2673	0.7659	
			ecotype:rel_habitat	4	2.18	0.545	0.4913	0.7421	
			Residuals	128	141.979	1.10921			
		Crab ecotype	rel_habitat	2	1.073	0.53633	0.3124	0.7342	
			Residuals	28	48.069	1.71674			
		Wave ecotype	rel_habitat	2	1.672	0.83585	0.7773	0.465	
			Residuals	51	54.843	1.07536			

		Hybrid ecotype	rel_habitat	2	0.029	0.01429	0.0179	0.9822	
			Residuals	49	39.067	0.79729			
		Crab release habitat	ecotype	2	2.4575	1.2287	2.1542	0.1325	
			Residuals	32	18.2527	0.5704			
		Wave release habitat	ecotype	2	0.3022	0.15109	0.1977	0.8219	
			Residuals	24	18.3416	0.76423			
		Hybrid release habitat	ecotype	2	1.921	0.96041	0.6562	0.5219	
			Residuals	72	105.385	1.46368			
Abbreviations: Recap_yn: recapture success; rel_habitat: release habitat ****: p<0.001; **: p<0.01; *: p<0.05; .:p<0.1*									

Supplementary Table 2. ANOVA outputs from general linear models of the effect of ecotype and release habitat (for all inversions, and sex (for inversions on LG12 only) on inversion genotype frequencies in various datasets. Abbreviations legend at the bottom of the table.

Site	Inversion	Dataset	Variable	DF	Sum sq	Mean sq	F value	Pr(>F)	Significance
ANG	LGC6.1	Full	NULL			8	17.426		
			ecotype2	2	15.855	6	1.571	0.0003607	***
			rel_habitat	2	0.24	4	1.331	0.8869236	
			ecotype2:rel_habitat	4	1.331	0	0	0.8560965	
		Crab ecotype	NULL			2	0.33252		
			rel_habitat	2	0.33252	0	0	0.8468	
		Wave ecotype	NULL	2	1.087	2	1.087		
			rel_habitat	2	1.087	0	0	0.5807	

		Hybrid ecotype	NULL			2	0.15148		
			rel_habitat	2	0.15148	0	0	0.9271	
		Crab release habitat	NULL			2	3.8079		
			ecotype2	2	3.8079	0	0	0.149	
		Wave release habitat	NULL	2	4.1243				
			ecotype2	2	4.1243	0	0	0.1272	
		Hybrid release habitat	NULL	2	9.0672				
			ecotype2	2	9.0672	0	0	0.01074	*
ANG	LGC6.2	Full	NULL			8	18.2015		
			ecotype2	2	16.9182	6	1.2833	0.000212	***
			rel_habitat	2	0.0906	4	1.1927	0.955712	
			ecotype2:rel_habitat	4	1.1927	0	0	0.879293	
		Crab ecotype	NULL	2	0.16479				
			rel_habitat	2	0.16479	0	0	0.9209	
		Wave ecotype	NULL			2	1.087		
			rel_habitat	2	1.087	0	0	0.5807	
		Hybrid ecotype	NULL			2	0.031558		
			rel_habitat	2	0.031558	0	0	0.9843	
		Crab release habitat	NULL			2	4.3773		
			ecotype2	2	4.3773	0	0	0.1121	

		Wave release habitat	NULL			2	3.7331		
			ecotype2	2	3.7331	0	0	0.1547	
		Hybrid release habitat	NULL			2	9.897		
			ecotype2	2	9.897	0	0	0.007094	**
ANG	LGC14.1	Full	NULL			8	38.472		
			ecotype2	2	32.762	6	5.71	7.69E-08	***
			rel_habitat	2	1.974	4	3.736	0.3727	
			ecotype2:rel_habitat	4	3.736	0	0	0.4429	
		Crab ecotype	NULL			2	0.23874		
			rel_habitat	2	0.23874	0	0	0.8875	
		Wave ecotype	NULL			2	4.6087		
			rel_habitat	2	4.6087	0	0	0.09982	.
		Hybrid ecotype	NULL			2	0.86239		
			rel_habitat	2	0.86239	0	0	0.6497	
		Crab release habitat	NULL			2	17.911		
			ecotype2	2	17.911	0	0	0.000129	***
		Wave release habitat	NULL			2	6.3946		
			ecotype2	2	6.3946	0	0	0.04087	*
		Hybrid release habitat	NULL			2	14.107		
			ecotype2	2	14.107	0	0	0.0008646	***
ANG	LGC14.2	Full	NULL			8	24.2169		

			ecotype2	2	21.4712	6	2.7458	2.18E-05	***
			rel_habitat	2	0.2948	4	2.451	0.8629	
			ecotype2:rel_habitat	4	2.451	0	0	0.6534	
		Crab ecotype	NULL			2	0.28514		
			rel_habitat	2	0.28514	0	0	0.8671	
		Wave ecotype	NULL			2	2.2198		
			rel_habitat	2	2.2198	0	0	0.3296	
		Hybrid ecotype	NULL			2	0.24086		
			rel_habitat	2	0.24086	0	0	0.8865	
		Crab release habitat	NULL	2	5.3748				
			ecotype2	2	5.3748	0	0	0.06806	.
		Wave release habitat	NULL			2	4.9207		
			ecotype2	2	4.9207	0	0	0.08541	.
		Hybrid release habitat	NULL			2	13.899		
			ecotype2	2	13.899	0	0	0.0009591	***
ANG	LGC14.3	Full	NULL			8	26.5029		
			ecotype2	2	25.3884	6	1.1145	3.07E-06	***
			rel_habitat	2	0.1051	4	1.0093	0.9488	
			ecotype2:rel_habitat	4	1.0093	0	0	0.9084	
		Crab ecotype	NULL			2	0.38974		
			rel_habitat	2	0.38974	0	0	0.8229	
		Wave ecotype	NULL			2	0.55981		
			rel_habitat	2	0.55981	0	0	0.7559	

		Hybrid ecotype	NULL			2	0.1649		
			rel_habitat	2	0.1649	0	0	0.9209	
		Crab release habitat	NULL			2	12.734		
			ecotype2	2	12.734	0	0	0.001717	**
		Wave release habitat	NULL			2	4.8113		
			ecotype2	2	4.8113	0	0	0.09021	.
		Hybrid release habitat	NULL			2	8.6367		
			ecotype2	2	8.6367	0	0	0.01332	*
ANG	LGC17.1	Full	NULL			8	74.86		
			ecotype2	2	65.896	6	8.964	4.91E-15	***
			rel_habitat	2	4.213	4	4.751	0.1217	
			ecotype2:rel_habitat	4	4.751	0	0	0.3138	
		Crab ecotype	NULL			2	0.00E+00		
			rel_habitat	2	0	0	2.96E-10	1	
		Wave ecotype	NULL			2	7.4107		
			rel_habitat	2	7.4107	0	0	0.02459	*
		Hybrid ecotype	NULL			2	1.5533		
			rel_habitat	2	1.5533	0	0	0.4599	
		Crab release habitat	NULL	2	18.72				
			ecotype2	2	18.72	0	0	8.61E-05	***

		Wave release habitat	NULL	2	9.193				
			ecotype2	2	9.193	0	0	0.01009	*
		Hybrid release habitat	NULL	2	40.732				
			ecotype2	2	40.732	0	0	1.43E-09	***
ANG	LGC12.2	Full	NULL			17	62.344		
			ecotype2	2	18.7449	15	43.599	8.50E-05	***
			rel_habitat	2	5.3726	13	38.226	0.068132	.
			sex	1	8.4159	12	29.81	0.003719	**
			ecotype2:rel_habitat	4	1.722	8	28.088	0.786716	
			ecotype2:sex	2	21.2985	6	6.79	2.37E-05	***
			rel_habitat:sex	2	0.3247	4	6.465	0.850147	
			ecotype2:rel_habitat:sex	4	6.465	0	0	0.167009	
		Crab ecotype	NULL			5	33.885		
			rel_habitat	2	3.6662	3	30.218	0.1599	
			sex	1	27.9572	2	2.261	1.24E-07	***
			rel_habitat:sex	2	2.2612	0	0	0.3228	
		Wave ecotype	NULL			5	6.2023		
			rel_habitat	2	3.00275	3	3.1995	0.2228	
			sex	1	0.44595	2	2.7536	0.5043	
			rel_habitat:sex	2	2.75357	0	0	0.2524	
		Hybrid ecotype	NULL			5	3.5119		
			rel_habitat	2	0.71793	3	2.794	0.6984	
			sex	1	1.01905	2	1.7749	0.3127	
			rel_habitat:sex	2	1.77493	0	0	0.4117	
		Crab release habitat	NULL			5	23.882		

			ecotype2	2	8.5386	3	15.344	0.013992	*
			sex	1	3.4894	2	11.854	0.061764	.
			ecotype2:sex	2	11.8542	0	0	0.002666	**
		Wave release habitat	NULL			5	14.2615		
			ecotype2	2	5.6354	3	8.6261	0.05974	.
			sex	1	2.8965	2	5.7295	0.08877	.
			ecotype2:sex	2	5.7295	0	0	0.057	.
		Hybrid release habitat	NULL	5	16.853				
			ecotype2	2	4.6108	3	12.2422	0.099719	.
			sex	1	2.2572	2	9.9851	0.132998	
			ecotype2:sex	2	9.9851	0	0	0.006788	**
ANG	LGC12.3	Full	NULL			17	61.39		
			ecotype2	2	18.0336	15	43.356	0.0001214	***
			rel_habitat	2	5.811	13	37.545	0.0547221	.
			sex	1	9.107	12	28.438	0.0025464	**
			ecotype2:rel_habitat	4	1.9441	8	26.494	0.7460381	
			ecotype2:sex	2	20.4108	6	6.083	3.70E-05	***
			rel_habitat:sex	2	0.2397	4	5.844	0.8870556	
			ecotype2:rel_habitat:sex	4	5.8437	0	0	0.2111296	
		Crab ecotype	NULL			5	33.424		
			rel_habitat	2	3.3759	3	30.048	0.1849	
			sex	1	27.793	2	2.255	1.35E-07	***
			rel_habitat:sex	2	2.2549	0	0	0.3239	
		Wave ecotype	NULL			5	6.427		
			rel_habitat	2	4.1059	3	2.3211	0.1284	
			sex	1	0.2196	2	2.1015	0.6393	
			rel_habitat:sex	2	2.1015	0	0	0.3497	

		Hybrid ecotype	NULL			5	3.5055		
			rel_habitat	2	0.71807	3	2.7874	0.6983	
			sex	1	1.06038	2	1.7271	0.3031	
			rel_habitat:sex	2	1.72706	0	0	0.4217	
		Crab release habitat	NULL			5	23.517		
			ecotype2	2	8.1797	3	15.337	0.016741	*
			sex	1	3.6546	2	11.682	0.055916	.
			ecotype2:sex	2	11.6825	0	0	0.002905	**
		Wave release habitat	NULL			5	14.1173		
			ecotype2	2	5.4912	3	8.6261	0.06421	.
			sex	1	2.9122	2	5.7139	0.08791	.
			ecotype2:sex	2	5.7139	0	0	0.05744	.
		Hybrid release habitat	NULL			5	15.4609		
			ecotype2	2	4.2676	3	11.1933	0.11839	
			sex	1	2.5756	2	8.6177	0.10852	
			ecotype2:sex	2	8.6177	0	0	0.01345	*
ANG	LGC12.4	Full	NULL			17	69.799		
			ecotype2	2	32.346	15	37.453	9.47E-08	***
			rel_habitat	2	3.18	13	34.273	0.20394	
			sex	1	6.082	12	28.191	0.01366	*
			ecotype2:rel_habitat	4	9.234	8	18.957	0.0555	.
			ecotype2:sex	2	7.428	6	11.529	0.02439	*
			rel_habitat:sex	2	0.461	4	11.068	0.79416	
			ecotype2:rel_habitat:sex	4	11.068	0	0	0.02581	*
		Crab ecotype	NULL			5	21.1762		
			rel_habitat	2	7.1616	3	14.0145	0.0278527	*

			sex	1	11.2958	2	2.7187	0.0007768	***
			rel_habitat:sex	2	2.7187	0	0	0.2568233	
		Wave ecotype	NULL			5	8.8191		
			rel_habitat	2	4.6854	3	4.1338	0.09607	.
			sex	1	1.0293	2	3.1044	0.31032	
			rel_habitat:sex	2	3.1044	0	0	0.21178	
		Hybrid ecotype	NULL			5	7.4578		
			rel_habitat	2	0.4624	3	6.9955	0.79359	
			sex	1	1.2893	2	5.7061	0.25617	
			rel_habitat:sex	2	5.7061	0	0	0.05767	.
		Crab release habitat	NULL			5	20.059		
			ecotype2	2	7.7265	3	12.333	0.021	*
			sex	1	1.9399	2	10.393	0.163684	
			ecotype2:sex	2	10.3929	0	0	0.005536	**
		Wave release habitat	NULL			5	15.796		
			ecotype2	2	6.7585	3	9.0375	0.03407	*
			sex	1	1.6011	2	7.4365	0.20575	
			ecotype2:sex	2	7.4365	0	0	0.02428	*
		Hybrid release habitat	NULL			5	33.203		
			ecotype2	2	29.4291	3	3.773	4.07E-07	***
			sex	1	2.6462	2	1.127	0.1038	
			ecotype2:sex	2	1.1272	0	0	0.5692	
CZB	LGC6.1	Full	NULL			8	44.508		
			ecotype2	2	39.21	6	5.298	3.06E-09	***
			rel_habitat	2	1.003	4	4.295	0.6057	
			ecotype2:rel_habitat	4	4.295	0	0	0.3675	

		Crab ecotype	NULL			2	0.44178		
			rel_habitat	2	0.44178	0	0	0.8018	
		Wave ecotype	NULL			2	4.2891		
			rel_habitat	2	4.2891	0	0	0.1171	
		Hybrid ecotype	NULL			2	0.56728		
			rel_habitat	2	0.56728	0	0	0.753	
		Crab release habitat	NULL			2	10.874		
			ecotype2	2	10.874	0	0	0.004352	**
		Wave release habitat	NULL			2	6.9517		
			ecotype2	2	6.9517	0	0	0.03094	*
		Hybrid release habitat	NULL			2	25.475		
			ecotype2	2	25.475	0	0	2.94E-06	***
CZB	LGC6.2	Full	NULL	8	43.205				
			ecotype2	2	37.966	6	5.239	5.70E-09	***
			rel_habitat	2	**1.156**	4	4.084	0.5611	
			ecotype2:rel_habitat	4	4.084	0	0	0.3948	
		Crab ecotype	NULL			2	0.44178		
			rel_habitat	2	0.44178	0	0	0.8018	
		Wave ecotype	NULL			2	4.3787		
			rel_habitat	2	4.3787	0	0	0.112	
		Hybrid ecotype	NULL			2	0.41887		
			rel_habitat	2	0.41887	0	0	0.811	

		Crab release habitat	NULL			2	9.964		
			ecotype2	2	9.964	0	0	0.00686	**
		Wave release habitat	NULL			2	6.2477		
			ecotype2	2	6.2477	0	0	0.04399	*
		Hybrid release habitat	NULL			2	25.614		
			ecotype2	2	25.614	0	0	2.74E-06	***
CZB	LGC14.1	Full	NULL			8	143.507		
			ecotype2	2	133.588	6	9.92	<0.0000000000000002	***
			rel_habitat	2	5.428	4	4.491	0.06626	.
			ecotype2:rel_habitat	4	4.491	0	0	0.3436	
		Crab ecotype	NULL			2	4.1922		
			rel_habitat	2	4.1922	0	0	0.1229	
		Wave ecotype	NULL			2	1.3728		
			rel_habitat	2	1.3728	0	0	0.5034	
		Hybrid ecotype	NULL			2	4.3546		
			rel_habitat	2	4.3546	0	0	0.1133	
		Crab release habitat	NULL			2	38.263		
			ecotype2	2	38.263	0	0	4.91E-09	***
		Wave release habitat	NULL			2	22.652		
			ecotype2	2	22.652	0	0	1.21E-05	***

		Hybrid release habitat	NULL			2	70.815		
			ecotype2	2	70.815	0	0	4.20E-16	***
CZB	LGC14.2	Full	NULL			8	11.8863		
			ecotype2	2	8.5238	6	3.3626	0.0141	*
			rel_habitat	2	1.3935	4	1.9691	0.4982	
			ecotype2:rel_habitat	4	1.9691	0	0	0.7414	
		Crab ecotype	NULL			2	2.5771		
			rel_habitat	2	2.5771	0	0	0.2757	
		Wave ecotype	NULL	2	0.2884				
			rel_habitat	2	0.2884	0	0	0.8657	
		Hybrid ecotype	NULL	2	0.49706				
			rel_habitat	2	0.49706	0	0	0.7799	
		Crab release habitat	NULL			2	2.9783		
			ecotype2	2	2.9783	0	0	0.2256	
		Wave release habitat	NULL	2	1.3623				
			ecotype2	2	1.3623	0	0	0.506	
		Hybrid release habitat	NULL			2	7.1205		
			ecotype2	2	7.1205	0	0	0.02843	*
CZB	LGC14.3	Full	NULL			8	30.2525		
			ecotype2	2	28.6245	6	1.628	6.09E-07	***
			rel_habitat	2	0.7599	4	0.8681	0.6839	
			ecotype2:rel_habitat	4	0.8681	0	0	0.9291	

		Crab ecotype	NULL	2	0.75981				
			rel_habitat	2	0.75981	0	0	0.6839	
		Wave ecotype	NULL			2	0.15183		
			rel_habitat	2	0.15183	0	0	0.9269	
		Hybrid ecotype	NULL			2	0.71639		
			rel_habitat	2	0.71639	0	0	0.6989	
		Crab release habitat	NULL			2	6.289		
			ecotype2	2	6.289	0	0	0.04309	*
		Wave release habitat	NULL			2	6.2989		
			ecotype2	2	6.2989	0	0	0.04288	*
		Hybrid release habitat	NULL			2	15.131		
			ecotype2	2	15.131	0	0	0.0005181	***
CZB	LGC17.1	Full	NULL			8	34.323		
			ecotype2	2	29.0652	6	5.258	4.88E-07	***
			rel_habitat	2	0.5577	4	4.7	0.7567	
			ecotype2:rel_habitat	4	4.6999	0	0	0.3195	
		Crab ecotype	NULL			2	0.00E+00		
			rel_habitat	2	0	0	2.98E-10	1	
		Wave ecotype	NULL			2	0.42307		
			rel_habitat	2	0.42307	0	0	0.8093	
		Hybrid ecotype	NULL			2	4.8345		
			rel_habitat	2	4.8345	0	0	0.08916	.

		Crab release habitat	NULL			2	7.4585		
			ecotype2	2	7.4585	0	0	0.02401	*
		Wave release habitat	NULL			2	12.966		
			ecotype2	2	12.966	0	0	0.00153	**
		Hybrid release habitat	NULL			2	13.765		
			ecotype2	2	13.765	0	0	0.001025	**
CZB	LGC12.2	Full	NULL			17	70.912		
			ecotype2	2	27.6854	15	43.226	9.73E-07	***
			rel_habitat	2	2.0462	13	41.18	0.359481	
			sex	1	7.3617	12	33.819	0.006663	**
			ecotype2:rel_habitat	4	2.1502	8	31.668	0.708161	
			ecotype2:sex	2	18.8781	6	12.79	7.96E-05	***
			rel_habitat:sex	2	2.7195	4	10.071	0.25672	
			ecotype2:rel_habitat:sex	4	10.0707	0	0	0.039253	*
		Crab ecotype	NULL			5	29.3908		
			rel_habitat	2	1.4406	3	27.9502	0.4866	
			sex	1	25.617	2	2.3333	4.16E-07	***
			rel_habitat:sex	2	2.3333	0	0	0.3114	
		Wave ecotype	NULL			5	3.9702		
			rel_habitat	2	0.2571	3	3.713	0.8793	
			sex	1	0.2406	2	3.4725	0.6238	
			rel_habitat:sex	2	3.4725	0	0	0.1762	
		Hybrid ecotype	NULL			5	9.8655		
			rel_habitat	2	2.7687	3	7.0968	0.25049	
			sex	1	0.1122	2	6.9846	0.73762	

			rel_habitat:sex	2	6.9846	0	0	0.03043	*
		Crab release habitat	NULL			5	25.842		
			ecotype2	2	11.0943	3	14.748	0.003898	**
			sex	1	3.8778	2	10.87	0.04893	*
			ecotype2:sex	2	10.8698	0	0	0.004362	**
		Wave release habitat	NULL			5	17.4999		
			ecotype2	2	7.7558	3	9.7441	0.02069	*
			sex	1	0.5576	2	9.1865	0.45523	
			ecotype2:sex	2	9.1865	0	0	0.01012	*
		Hybrid release habitat	NULL			5	25.1095		
			ecotype2	2	10.8411	3	14.2684	0.004425	**
			sex	1	6.7997	2	7.4687	0.009118	**
			ecotype2:sex	2	7.4687	0	0	0.023888	*
CZB	LGC12.3	Full	NULL			17	72.673		
			ecotype2	2	29.8243	15	42.848	3.34E-07	***
			rel_habitat	2	2.9219	13	39.926	0.232018	
			sex	1	7.2658	12	32.661	0.007028	**
			ecotype2:rel_habitat	4	2.2541	8	30.407	0.689133	
			ecotype2:sex	2	18.5317	6	11.875	9.46E-05	***
			rel_habitat:sex	2	2.4252	4	9.45	0.297427	
			ecotype2:rel_habitat:sex	4	9.4497	0	0	0.050792	.
		Crab ecotype	NULL			5	29.2354		
			rel_habitat	2	1.2683	3	27.9671	0.5304	
			sex	1	25.5985	2	2.3686	4.20E-07	***
			rel_habitat:sex	2	2.3686	0	0	0.306	
		Wave ecotype	NULL			5	4.1957		

			rel_habitat	2	0.1633	3	4.0325	0.9216	
			sex	1	0.1819	2	3.8505	0.6697	
			rel_habitat:sex	2	3.8505	0	0	0.1458	
		Hybrid ecotype	NULL			5	9.4172		
			rel_habitat	2	3.663	3	5.7541	0.16017	
			sex	1	0.0985	2	5.6557	0.75369	
			rel_habitat:sex	2	5.6557	0	0	0.05914	.
		Crab release habitat	NULL			5	25.842		
			ecotype2	2	11.0943	3	14.748	0.003898	**
			sex	1	3.8778	2	10.87	0.04893	*
			ecotype2:sex	2	10.8698	0	0	0.004362	**
		Wave release habitat	NULL			5	17.98		
			ecotype2	2	8.6216	3	9.3583	0.01342	*
			sex	1	0.3794	2	8.979	0.53793	
			ecotype2:sex	2	8.979	0	0	0.01123	*
		Hybrid release habitat	NULL			5	24.8928		
			ecotype2	2	11.245	3	13.6478	0.003616	**
			sex	1	6.4498	2	7.1979	0.011096	*
			ecotype2:sex	2	7.1979	0	0	0.027352	*
CZB	LGC12.4	Full	NULL			17	66.895		
			ecotype2	2	40.385	15	26.51	1.70E-09	***
			rel_habitat	2	8.808	13	17.702	0.01223	*
			sex	1	0.007	12	17.695	0.93291	
			ecotype2:rel_habitat	4	2.866	8	14.829	0.58054	
			ecotype2:sex	2	1.545	6	13.285	0.46193	
			rel_habitat:sex	2	7.435	4	5.849	0.02429	*
			ecotype2:rel_habitat:sex	4	5.849	0	0	0.21068	

		Crab ecotype	NULL			5	11.5301		
			rel_habitat	2	9.2161	3	2.314	0.009971	**
			sex	1	0.8568	2	1.4572	0.354644	
			rel_habitat:sex	2	1.4572	0	0	0.482585	
		Wave ecotype	NULL			5	9.9623		
			rel_habitat	2	0.6143	3	9.348	0.73555	
			sex	1	0.3531	2	8.9949	0.55239	
			rel_habitat:sex	2	8.9949	0	0	0.01114	*
		Hybrid ecotype	NULL			5	5.0177		
			rel_habitat	2	1.84087	3	3.1768	0.3983	
			sex	1	0.34441	2	2.8324	0.5573	
			rel_habitat:sex	2	2.83241	0	0	0.2426	
		Crab release habitat	NULL			5	10.8858		
			ecotype2	2	4.9601	3	5.9257	0.08374	.
			sex	1	3.1925	2	2.7332	0.07398	.
			ecotype2:sex	2	2.7332	0	0	0.25497	
		Wave release habitat	NULL			5	14.1855		
			ecotype2	2	9.6672	3	4.5182	0.007958	**
			sex	1	1.5139	2	3.0043	0.218542	
			ecotype2:sex	2	3.0043	0	0	0.222648	
		Hybrid release habitat	NULL			5	38.975		
			ecotype2	2	34.58	3	4.395	3.10E-08	***
			sex	1	3.207	2	1.188	0.07331	.
			ecotype2:sex	2	1.188	0	0	0.55224	
Abbreviations: Recap_yn: recapture success; rel_habitat: release habitat; ecotype2: ecotype									

****: p<0.001; **: p<0.01; *: p<0.05; .:p<0.1*

Supplementary Table 3. ANOVA outputs from general linear models of the effect of ecotype, release habitat, and recapture rate on movement metrics in various datasets. Abbreviations legend at the bottom of the table.

Site	Movement metric	Dataset	Variable	DF	Sum sq	Mean sq	F value	Pr(>F)	Significance
ANG	Along-shore	Full	recap_yn	1	0.0117	0.011719	0.8113	0.36825	
			ecotype	2	0.1293	0.064631	4.4743	0.01195	*
			rel_habitat	2	0.0779	0.038969	2.6977	0.06854	.
			recap_yn:ecotype	2	0.0366	0.018307	1.2674	0.28265	
			recap_yn:rel_habitat	2	0.0451	0.02253	1.5597	0.21143	
			ecotype:rel_habitat	4	0.0549	0.013725	0.9502	0.43482	
			recap_yn:ecotype:rel_habitat	4	0.0737	0.018435	1.2762	0.27859	
			Residuals	416	6.009	0.014445			
		Crab ecotype	recap_yn	1	0.05579	0.055789	3.9803	0.04799	*
			rel_habitat	2	0.02218	0.01109	0.7912	0.45532	
			recap_yn:rel_habitat	2	0.01008	0.005039	0.3595	0.69866	
			Residuals	139	1.94825	0.014016			
		Wave ecotype	recap_yn	1	0.00173	0.001732	0.1019	0.7501	
			rel_habitat	2	0.06842	0.034209	2.0117	0.138	
			recap_yn:rel_habitat	2	0.07673	0.038365	2.2561	0.1089	
			Residuals	127	2.1596	0.017005			
		Hybrid ecotype	recap_yn	1	0.00092	0.0009155	0.0722	0.7885	
			rel_habitat	2	0.02691	0.0134554	1.0616	0.3485	
			recap_yn:rel_habitat	2	0.03408	0.0170394	1.3444	0.2638	
			Residuals	150	1.9012	0.0126747			

		Crab release habitat	recap_yn	1	0.02794	0.02794	1.491	0.2254	
			ecotype	2	0.01351	0.006755	0.3605	0.6984	
			recap_yn:ecotype	2	0.03794	0.018971	1.0124	0.3676	
			Residuals	87	1.63024	0.018738			
		Wave release habitat	recap_yn	1	0.00614	0.0061424	0.4842	0.488	
			ecotype	2	0.00855	0.0042763	0.3371	0.7146	
			recap_yn:ecotype	2	0.05654	0.0282694	2.2283	0.1125	
			Residuals	112	1.42089	0.0126865			
		Hybrid release habitat	recap_yn	1	0.02764	0.027641	2.0278	0.155878	
			ecotype	2	0.15916	0.079581	5.8383	0.003391	**
			recap_yn:ecotype	2	0.01533	0.007667	0.5625	0.570621	
			Residuals	217	2.95791	0.013631			
CZB	Along-shore	Full	recap_yn	1	0.00854	0.0085391	1.2744	0.25953	
			ecotype	2	0.00537	0.0026853	0.4008	0.67005	
			rel_habitat	2	0.00262	0.0013112	0.1957	0.82233	
			recap_yn:ecotype	2	0.01529	0.0076432	1.1407	0.3205	
			recap_yn:rel_habitat	2	0.0095	0.0047501	0.7089	0.49272	
			ecotype:rel_habitat	4	0.0062	0.0015496	0.2313	0.92079	
			recap_yn:ecotype:rel_habitat	4	0.05623	0.0140573	2.0979	0.08007	.
			Residuals	463	3.10239	0.0067006			
		Crab ecotype	recap_yn	1	0.00064	0.0006378	0.0853	0.7706	
			rel_habitat	2	0.00105	0.0005273	0.0705	0.9319	
			recap_yn:rel_habitat	2	0.00128	0.0006389	0.0855	0.9181	
			Residuals	151	1.12874	0.0074751			
		Wave ecotype	recap_yn	1	0.00169	0.0016866	0.4313	0.5124096	

			rel_habitat	2	0.0044	0.002199	0.5623	0.5711469	
			recap_yn:rel_habitat	2	0.06085	0.0304253	7.7802	0.0006203	***
			Residuals	143	0.55921	0.0039106			
		Hybrid ecotype	recap_yn	1	0.02237	0.022372	2.6731	0.1039	
			rel_habitat	2	0.00527	0.0026326	0.3146	0.7305	
			recap_yn:rel_habitat	2	0.00202	0.001011	0.1208	0.8863	
			Residuals	169	1.41443	0.0083694			
		Crab release habitat	recap_yn	1	0.00088	0.0008807	0.2385	0.626447	
			ecotype	2	0.00449	0.0022426	0.6072	0.546979	
			recap_yn:ecotype	2	0.04977	0.0248838	6.7379	0.001843	**
			Residuals	94	0.34715	0.0036931			
		Wave release habitat	recap_yn	1	0.01353	0.013525	1.7112	0.1935	
			ecotype	2	0.00186	0.0009275	0.1174	0.8894	
			recap_yn:ecotype	2	0.01787	0.0089329	1.1302	0.3266	
			Residuals	112	0.88521	0.0079037			
		Hybrid release habitat	recap_yn	1	0.00501	0.0050112	0.6887	0.4074	
			ecotype	2	0.00372	0.0018615	0.2558	0.7745	
			recap_yn:ecotype	2	0.00446	0.0022315	0.3067	0.7362	
			Residuals	257	1.87002	0.0072763			
ANG	Seaward	Full	recap_yn	1	0.00534	0.005344	1.5898	0.20806	
			ecotype	2	0.00427	0.0021327	0.6345	0.53073	
			rel_habitat	2	0.0195	0.0097521	2.9012	0.05607	.
			recap_yn:ecotype	2	0.02289	0.0114447	3.4047	0.03414	*
			recap_yn:rel_habitat	2	0.02213	0.011066	3.2921	0.03815	*
			ecotype:rel_habitat	4	0.02857	0.0071429	2.125	0.0769	.
			recap_yn:ecotype:rel_habitat	4	0.0174	0.0043511	1.2944	0.27145	
			Residuals	416	1.39835	0.0033614			

		Crab ecotype	recap_yn	1	0.001427	0.0014265	0.7441	0.389848	
			rel_habitat	2	0.024497	0.0122483	6.3885	0.002217	**
			recap_yn:rel_habitat	2	0.014628	0.0073141	3.8149	0.024382	*
			Residuals	139	0.266496	0.0019172			
		Wave ecotype	recap_yn	1	0.000035	0.0000353	0.0144	0.90453	
			rel_habitat	2	0.013809	0.0069047	2.8217	0.06324	.
			recap_yn:rel_habitat	2	0.002617	0.0013086	0.5348	0.58711	
			Residuals	127	0.310762	0.0024469			
		Hybrid ecotype	recap_yn	1	0.0287	0.0286998	5.243	0.02343	*
			rel_habitat	2	0.00318	0.0015899	0.2904	0.74835	
			recap_yn:rel_habitat	2	0.02812	0.014061	2.5687	0.08	.
			Residuals	150	0.82109	0.0054739			
		Crab release habitat	recap_yn	1	0.03041	0.0304093	2.4578	0.1206	
			ecotype	2	0.02079	0.0103931	0.84	0.4352	
			recap_yn:ecotype	2	0.01714	0.0085679	0.6925	0.5031	
			Residuals	87	1.07643	0.0123728			
		Wave release habitat	recap_yn	1	0.000091	0.0000905	0.0455	0.83145	
			ecotype	2	0.005658	0.0028289	1.422	0.24555	
			recap_yn:ecotype	2	0.017876	0.0089379	4.4928	0.01328	*
			Residuals	112	0.22281	0.0019894			
		Hybrid release habitat	recap_yn	1	0.000005	0.0000047	0.0103	0.9191344	
			ecotype	2	0.008408	0.0042039	9.205	0.0001455	***
			recap_yn:ecotype	2	0.001321	0.0006604	1.4461	0.2377403	
			Residuals	217	0.099104	0.0004567			
CZB	Seaward	Full	recap_yn	1	0.0012	0.0011974	0.2669	0.60566	

			ecotype	2	0.03571	0.0178536	3.9798	0.01933	*
			rel_habitat	2	0.01501	0.0075031	1.6725	0.18891	
			recap_yn:ecotype	2	0.01856	0.0092799	2.0686	0.12753	
			recap_yn:rel_habitat	2	0.01165	0.0058226	1.2979	0.27409	
			ecotype:rel_habitat	4	0.04598	0.0114952	2.5624	0.03782	*
			recap_yn:ecotype:rel_habitat	4	0.02028	0.0050708	1.1303	0.34147	
			Residuals	463	2.07706	0.0044861			
		Crab ecotype	recap_yn	1	0.00276	0.0027631	0.6092	0.4363	
			rel_habitat	2	0.00789	0.0039446	0.8696	0.4212	
			recap_yn:rel_habitat	2	0.00709	0.0035465	0.7819	0.4594	
			Residuals	151	0.68492	0.0045359			
		Wave ecotype	recap_yn	1	0.00765	0.0076514	2.1253	0.1471	
			rel_habitat	2	0.00703	0.0035142	0.9761	0.3793	
			recap_yn:rel_habitat	2	0.01645	0.0082261	2.285	0.1055	
			Residuals	143	0.51481	0.0036001			
		Hybrid ecotype	recap_yn	1	0.00969	0.0096936	1.8673	0.1736	
			rel_habitat	2	0.04835	0.0241734	4.6565	0.01075	*
			recap_yn:rel_habitat	2	0.00493	0.0024672	0.4753	0.62255	
			Residuals	169	0.87733	0.0051913			
		Crab release habitat	recap_yn	1	0.00728	0.0072769	1.6537	0.20161	
			ecotype	2	0.01091	0.0054561	1.2399	0.29409	
			recap_yn:ecotype	2	0.0354	0.017698	4.022	0.02109	*
			Residuals	94	0.41363	0.0044003			
		Wave release habitat	recap_yn	1	0.00404	0.004037	0.6769	0.412396	
			ecotype	2	0.06375	0.031873	5.3444	0.006069	**
			recap_yn:ecotype	2	0.00095	0.000473	0.0792	0.923861	
			Residuals	112	0.66795	0.005964			

		Hybrid release habitat	recap_yn	1	0.00097	0.0009734	0.2513	0.6166	
			ecotype	2	0.00467	0.0023351	0.6028	0.548	
			recap_yn:ecotype	2	0.00422	0.0021124	0.5453	0.5803	
			Residuals	257	0.99548	0.0038735			
ANG	Vertical	Full	recap_yn	1	0.00549	0.005488	1.5906	0.207949	
			ecotype	2	0.00058	0.00029	0.0842	0.919274	
			rel_habitat	2	0.14241	0.071207	20.6378	2.84E-09	***
			recap_yn:ecotype	2	0.01831	0.009156	2.6537	0.071579	.
			recap_yn:rel_habitat	2	0.04407	0.022036	6.3867	0.001853	**
			ecotype:rel_habitat	4	0.03878	0.009694	2.8096	0.025287	*
			recap_yn:ecotype:rel_habitat	4	0.02911	0.007278	2.1093	0.078839	.
			Residuals	417	1.43878	0.00345			
		Crab ecotype	recap_yn	1	0.01472	0.0147223	4.0067	0.047265	*
			rel_habitat	2	0.04906	0.024528	6.6754	0.001705	**
			recap_yn:rel_habitat	2	0.02424	0.0121218	3.299	0.039831	*
			Residuals	139	0.51074	0.0036744			
		Wave ecotype	recap_yn	1	0.00354	0.003543	0.9231	0.338485	
			rel_habitat	2	0.12493	0.062464	16.2732	5.11E-07	***
			recap_yn:rel_habitat	2	0.04145	0.020725	5.3993	0.005617	**
			Residuals	127	0.48748	0.003838			
		Hybrid ecotype	recap_yn	1	0.00253	0.002526	0.8658	0.35361	
			rel_habitat	2	0.01525	0.0076229	2.6127	0.07665	.
			recap_yn:rel_habitat	2	0.0018	0.0009022	0.3092	0.73448	
			Residuals	151	0.44056	0.0029176			
		Crab release habitat	recap_yn	1	0.00141	0.0014072	0.3357	0.5638	
			ecotype	2	0.01568	0.0078375	1.8697	0.1603	
			recap_yn:ecotype	2	0.00653	0.0032642	0.7787	0.4622	

			Residuals	87	0.3647	0.004192			
		Wave release habitat	recap_yn	1	0.04518	0.045184	5.6477	0.01918	*
			ecotype	2	0.00897	0.004486	0.5608	0.57237	
			recap_yn:ecotype	2	0.02881	0.014403	1.8003	0.16999	
			Residuals	112	0.89605	0.008			
		Hybrid release habitat	recap_yn	1	0.004988	0.0049885	6.1085	0.01422	*
			ecotype	2	0.01803	0.0090151	11.0392	2.71E-05	***
			recap_yn:ecotype	2	0.007428	0.0037139	4.5478	0.01161	*
			Residuals	218	0.178028	0.0008166			
CZB	Vertical	Full	recap_yn	1	0.001213	0.0012129	2.1518	0.143063	
			ecotype	2	0.007904	0.0039522	7.0115	0.000998	***
			rel_habitat	2	0.002885	0.0014427	2.5594	0.078419	.
			recap_yn:ecotype	2	0.001942	0.0009711	1.7229	0.179671	
			recap_yn:rel_habitat	2	0.000323	0.0001616	0.2867	0.750849	
			ecotype:rel_habitat	4	0.004165	0.0010412	1.8471	0.118647	
			recap_yn:ecotype:rel_habitat	4	0.001967	0.0004918	0.8724	0.480267	
			Residuals	474	0.267181	0.0005637			
		Crab ecotype	recap_yn	1	0.000122	0.00012183	0.2046	0.6517	
			rel_habitat	2	0.002369	0.00118458	1.9893	0.1403	
			recap_yn:rel_habitat	2	0.000038	0.00001884	0.0316	0.9689	
			Residuals	155	0.0923	0.00059548			
		Wave ecotype	recap_yn	1	0.000898	0.00089773	1.261	0.2633	
			rel_habitat	2	0.000861	0.00043055	0.6048	0.5475	
			recap_yn:rel_habitat	2	0.000501	0.00025041	0.3517	0.704	
			Residuals	148	0.105361	0.0007119			
		Hybrid ecotype	recap_yn	1	0.002391	0.00239053	5.88	0.01635	*
			rel_habitat	2	0.00383	0.00191521	4.7109	0.0102	*

			recap_yn:rel_habitat	2	0.001856	0.0009282	2.2831	0.10507	
			Residuals	171	0.069521	0.00040655			
		Crab release habitat	recap_yn	1	0.000952	0.00095226	2.877	0.0931	.
			ecotype	2	0.002023	0.00101142	3.0557	0.0517	.
			recap_yn:ecotype	2	0.002495	0.00124766	3.7694	0.02655	*
			Residuals	96	0.031776	0.000331			
		Wave release habitat	recap_yn	1	0.000584	0.0005836	0.7103	0.401055	
			ecotype	2	0.008389	0.0041946	5.1053	0.007486	**
			recap_yn:ecotype	2	0.000852	0.0004262	0.5188	0.596614	
			Residuals	117	0.096129	0.0008216			
		Hybrid release habitat	recap_yn	1	0.000386	0.00038625	0.7238	0.3957	
			ecotype	2	0.002415	0.0012074	2.2626	0.1061	
			recap_yn:ecotype	2	0.000471	0.00023568	0.4417	0.6435	
			Residuals	261	0.139277	0.00053363			
ANG	Distance from release	Full	recap_yn	1	54.33	54.328	33.649	1.31E-08	***
			ecotype	2	5.14	2.57	1.5915	0.20485	
			rel_habitat	2	72.8	36.402	22.5458	5.04E-10	***
			recap_yn:ecotype	2	7.91	3.956	2.4505	0.08749	.
			recap_yn:rel_habitat	2	6.18	3.089	1.9133	0.14888	
			ecotype:rel_habitat	4	9.26	2.315	1.4341	0.22181	
			recap_yn:ecotype:rel_habitat	4	6.03	1.508	0.934	0.44406	
			Residuals	417	673.27	1.615			
		Crab ecotype	recap_yn	1	0.22383	0.223833	17.4908	5.09E-05	***
			rel_habitat	2	0.03708	0.018538	1.4486	0.2384	
			recap_yn:rel_habitat	2	0.02165	0.010826	0.846	0.4313	

			Residuals	139	1.77881	0.012797			
		Wave ecotype	recap_yn	1	0.08877	0.088769	7.2711	0.007958	**
			rel_habitat	2	0.37514	0.187571	15.364	1.06E-06	***
			recap_yn:rel_habitat	2	0.1053	0.052652	4.3128	0.015412	*
			Residuals	127	1.55048	0.012209			
		Hybrid ecotype	recap_yn	1	6.28	6.2838	1.4401	0.232	
			rel_habitat	2	3.16	1.5798	0.3621	0.6968	
			recap_yn:rel_habitat	2	4.2	2.1013	0.4816	0.6188	
			Residuals	151	658.88	4.3635			
		Crab release habitat	recap_yn	1	0.47901	0.47901	35.106	6.11E-08	***
			ecotype	2	0.0845	0.04225	3.0963	0.05023	.
			recap_yn:ecotype	2	0.01277	0.00639	0.4681	0.62777	
			Residuals	87	1.18708	0.01364			
		Wave release habitat	recap_yn	1	0.13967	0.13967	10.3358	0.001705	**
			ecotype	2	0.02301	0.011507	0.8515	0.429502	
			recap_yn:ecotype	2	0.0573	0.02865	2.1201	0.124808	
			Residuals	112	1.51347	0.013513			
		Hybrid release habitat	recap_yn	1	2.67	2.6728	0.8835	0.3483	
			ecotype	2	7.57	3.7861	1.2515	0.2881	
			recap_yn:ecotype	2	5.53	2.7666	0.9145	0.4022	
			Residuals	218	659.51	3.0253			
CZB	Distance from release	Full	recap_yn	1	28.07	28.0685	21.127	0.000005519	***
			ecotype	2	1.56	0.7814	0.5882	0.55574	
			rel_habitat	2	12.69	6.3473	4.7776	0.008826	**

			recap_yn:ecotype	2	5.41	2.706	2.0368	0.131585	
			recap_yn:rel_habitat	2	16.09	8.0447	6.0552	0.002531	**
			ecotype:rel_habitat	4	14.53	3.633	2.7345	0.028463	*
			recap_yn:ecotype:rel_habitat	4	1.78	0.4461	0.3358	0.853874	
			Residuals	474	629.74	1.3286			
		Crab ecotype	recap_yn	1	0.04008	0.040082	5.7827	0.01736	*
			rel_habitat	2	0.03497	0.017487	2.5228	0.08352	.
			recap_yn:rel_habitat	2	0.00413	0.002065	0.2979	0.7428	
			Residuals	155	1.07436	0.006931			
		Wave ecotype	recap_yn	1	0.421	0.42145	0.9273	0.3371	
			rel_habitat	2	0.196	0.09796	0.2155	0.8064	
			recap_yn:rel_habitat	2	0.5	0.24983	0.5497	0.5783	
			Residuals	148	67.262	0.45447			
		Hybrid ecotype	recap_yn	1	0.3538	0.35381	6.3202	0.01286	*
			rel_habitat	2	0.0118	0.00589	0.1051	0.90026	
			recap_yn:rel_habitat	2	0.0827	0.04135	0.7387	0.47927	
			Residuals	171	9.5727	0.05598			
		Crab release habitat	recap_yn	1	0.08167	0.081674	17.6335	5.99E-05	***
			ecotype	2	0.02306	0.011528	2.489	0.08834	.
			recap_yn:ecotype	2	0.01599	0.007996	1.7263	0.18342	
			Residuals	96	0.44465	0.004632			
		Wave release habitat	recap_yn	1	0.00773	0.007734	0.6616	0.4176	
			ecotype	2	0.01862	0.0093104	0.7964	0.4534	
			recap_yn:ecotype	2	0.04001	0.0200072	1.7115	0.1851	
			Residuals	117	1.36772	0.0116899			

		Hybrid release habitat	recap_yn	1	0.934	0.93363	3.2022	0.0747	.
			ecotype	2	0.267	0.13346	0.4577	0.6332	
			recap_yn:ecotype	2	0.279	0.13967	0.4791	0.6199	
			Residuals	261	76.097	0.29156			
ANG	Distance moved	Full	recap_yn	1	42.7	42.698	33.0387	1.75E-08	***
			ecotype	2	4.66	2.331	1.804	0.16592	
			rel_habitat	2	55.73	27.866	21.562	1.23E-09	***
			recap_yn:ecotype	2	4.3	2.148	1.6621	0.191	
			recap_yn:rel_habitat	2	6.45	3.224	2.4947	0.08376	.
			ecotype:rel_habitat	4	7.42	1.854	1.4344	0.2217	
			recap_yn:ecotype:rel_habitat	4	3.41	0.852	0.6589	0.6209	
			Residuals	417	538.92	1.292			
		Crab ecotype	recap_yn	1	0.24303	0.243034	14.376	0.0002224	***
			rel_habitat	2	0.04128	0.020641	1.221	0.2980847	
			recap_yn:rel_habitat	2	0.00608	0.003042	0.18	0.835488	
			Residuals	139	2.34984	0.016905			
		Wave ecotype	recap_yn	1	0.09275	0.092746	6.5501	0.01166	*
			rel_habitat	2	0.33923	0.169615	11.979	1.72E-05	***
			recap_yn:rel_habitat	2	0.08444	0.042222	2.9819	0.05426	.
			Residuals	127	1.79825	0.014159			
		Hybrid ecotype	recap_yn	1	6.21	6.2058	1.423	0.2348	
			rel_habitat	2	3.17	1.5851	0.3635	0.6959	
			recap_yn:rel_habitat	2	4.18	2.0887	0.4789	0.6204	
			Residuals	151	658.52	4.3611			
		Crab release habitat	recap_yn	1	0.45451	0.45451	32.2631	1.75E-07	***
			ecotype	2	0.07481	0.03741	2.6553	0.07597	.

			recap_yn:ecotype	2	0.00764	0.00382	0.2711	0.76317	
			Residuals	87	1.22561	0.01409			
		Wave release habitat	recap_yn	1	0.11795	0.117949	8.2791	0.004803	**
			ecotype	2	0.02123	0.010614	0.745	0.477052	
			recap_yn:ecotype	2	0.03733	0.018663	1.31	0.273913	
			Residuals	112	1.59561	0.014247			
		Hybrid release habitat	recap_yn	1	2.81	2.8125	0.9292	0.3361	
			ecotype	2	7.45	3.7234	1.2301	0.2943	
			recap_yn:ecotype	2	5.32	2.6603	0.8789	0.4167	
			Residuals	218	659.85	3.0268			
CZB	Distance moved	Full	recap_yn	1	22.13	22.1279	15.8388	0.00007983	***
			ecotype	2	4.92	2.4605	1.7612	0.1729741	
			rel_habitat	2	25.47	12.7337	9.1146	0.0001307	***
			recap_yn:ecotype	2	7.88	3.939	2.8195	0.0606421	.
			recap_yn:rel_habitat	2	11.36	5.6785	4.0646	0.0177748	*
			ecotype:rel_habitat	4	12.17	3.0425	2.1778	0.070469	.
			recap_yn:ecotype:rel_habitat	4	4.88	1.2209	0.8739	0.4793881	
			Residuals	472	659.42	1.3971			
		Crab ecotype	recap_yn	1	5.65	5.6465	1.3099	0.25418	
			rel_habitat	2	28.39	14.1957	3.2932	0.03975	*
			recap_yn:rel_habitat	2	12.09	6.0429	1.4019	0.24924	
			Residuals	155	668.14	4.3106			
		Wave ecotype	recap_yn	1	0.347	0.3468	0.1978	0.6572	
			rel_habitat	2	2.023	1.0116	0.5768	0.5629	
			recap_yn:rel_habitat	2	6.859	3.4297	1.9555	0.1451	
			Residuals	148	259.568	1.7538			

		Hybrid ecotype	recap_yn	1	0.3633	0.36332	6.3848	0.01242	*
			rel_habitat	2	0.0231	0.01156	0.2032	0.81633	
			recap_yn:rel_habitat	2	0.0867	0.04337	0.7621	0.46825	
			Residuals	171	9.7306	0.0569			
		Crab release habitat	recap_yn	1	0.05903	0.059031	7.4019	0.007736	**
			ecotype	2	0.05607	0.028033	3.5151	0.033631	*
			recap_yn:ecotype	2	0.02475	0.012377	1.552	0.217095	
			Residuals	96	0.76561	0.007975			
		Wave release habitat	recap_yn	1	0.9	0.902	0.1226	0.7268	
			ecotype	2	19.65	9.827	1.3362	0.2668	
			recap_yn:ecotype	2	22.13	11.0656	1.5046	0.2264	
			Residuals	117	860.47	7.3545			
		Hybrid release habitat	recap_yn	1	0.932	0.93192	3.1918	0.07517	.
			ecotype	2	0.257	0.12847	0.44	0.6445	
			recap_yn:ecotype	2	0.313	0.15642	0.5357	0.58587	
			Residuals	261	76.204	0.29197			
Abbreviations: Recap_yn: recapture success; rel_habitat: release habitat ****: p<0.001; **: p<0.01; *: p<0.05; .:p<0.1*									

Supplementary Table 4. Chi-squared test outputs from general linear models of the effect of ecotype, release habitat, and recapture rate on recapture rate in various datasets. Abbreviations legend at the bottom of the table.

Site	Dataset	Variable	DF	Deviance	Residual DF	Residual deviance	Pr(>Chi)	Significance
ANG	Full	NULL			8	22.3019		
		ecotype	2	15.5436	6	6.7583	0.0004215	***

		rel_habitat	2	0.9611	4	5.7972	0.6184416	
		ecotype:rel_habitat	4	5.7972	0	0	0.2148135	
	Crab ecotype	NULL			2	5.9365		
		rel_habitat	2	5.9365	0	0	0.05139	.
	Wave ecotype	NULL			2	0.66714		
		rel_habitat	2	0.66714	0	0	0.7164	
	Hybrid ecotype	NULL			2	0.15469		
		rel_habitat	2	0.15469	0	0	0.9256	
	Crab release habitat	NULL			2	7.2816		
		ecotype	2	7.2816	0	0	0.02623	*
	Wave release habitat	NULL			2	3.4034		
		ecotype	2	3.4034	0	0	0.1824	
	Hybrid release habitat	NULL			2	10.703		
		ecotype	2	10.703	0	0	0.00474	**
CZB	Full	NULL			8	27.572		
		ecotype	2	8.2989	6	19.273	0.015773	*
		rel_habitat	2	1.6051	4	17.668	0.448183	
		ecotype:rel_habitat	4	17.6679	0	0	0.001433	**
	Crab ecotype	NULL			2	2.2297		
		rel_habitat	2	2.2297	0	0	0.328	
	Wave ecotype	NULL			2	9.7718		
		rel_habitat	2	9.7718	0	0	0.007552	**

	Hybrid ecotype	NULL			2	7.2716		
		rel_habitat	2	7.2716	0	0	0.02636	*
	Crab release habitat	NULL			2	22.977		
		ecotype	2	22.977	0	0	1.02E-05	***
	Wave release habitat	NULL			2	2.0134		
		ecotype	2	2.0134	0	0	0.3654	
	Hybrid release habitat	NULL			2	0.99578		
		ecotype	2	0.99578	0	0	0.6078	
Abbreviations: Recap_yn: recapture success; rel_habitat: release habitat ****: p<0.001; **: p<0.01; *: p<0.05; .:p<0.1*								

Supplementary Table 5. RMark model selection tables for all model sets tested (A1-5, B, C, D1, D2) at both sites. Abbreviations legend at the bottom of the table.

Site	Model set	Dataset	Model	Npar	AICc	DeltaAICc	Weight	Deviance
ANG	A1	Full	Phi(~ecotype*rel_hab+time)p(~1)	25	2415.088	0	0.969975000	1393.251
			Phi(~ecotype+time)p(~1)	19	2422.059	6.971211	0.029715390	1412.67
			Phi(~time)p(~1)	17	2432.373	17.284978	0.000171149	1427.106
			Phi(~rel_hab+time)p(~1)	19	2432.798	17.709911	0.000138389	1423.409

			Phi(~ecotype*rel_hab)p(~1)	10	2.45E+03	32.981467	6.68E-08	1457.124
			Phi(~ecotype)p(~1)	4	2.45E+03	38.329448	4.61E-09	1474.619
			Phi(~1)p(~1)	2	2464.255	49.166561	2.04E-11	1489.479
			Phi(~rel_hab)p(~1)	4	2464.782	49.693348	1.57E-11	1485.983
		Crab release habitat	Phi(~ecotype+time)p(~1)	19	665.0085	0	0.635578500	399.247
			Phi(~ecotype)p(~1)	4	666.1538	1.145251	0.358493300	433.5062
			Phi(~1)p(~1)	2	674.3888	9.380211	0.005838246	445.8549
			Phi(~time)p(~1)	17	682.7361	17.727593	0.000089882	421.6351
		Wave release habitat	Phi(~ecotype+time)p(~1)	19	551.4019	0	0.8505067	298.7933
			Phi(~1)p(~1)	2	556.3335	4.931622	0.072241990	340.2204
			Phi(~time)p(~1)	17	556.8821	5.480237	0.054911010	308.7804
			Phi(~ecotype)p(~1)	4	5.59E+02	7.278881	0.022340290	338.4785
		Hybrid release	Phi(~ecotype+time)p(~1)	19	1116.785	0	0.522851847	543.4067

		habitat						
			Phi(~time)p(~1)	17	1117.06	0.275099	0.455660799	547.9131
			Phi(~ecotype)p(~1)	4	1123.264	6.478892	0.020488259	580.9981
			Phi(~1)p(~1)	2	1129.305	12.520406	0.000999096	591.0819
		Crab ecotype	Phi(~rel_hab+time)p(~1)	19	825.7458	0	0.512866543	469.52
			Phi(~rel_hab)p(~1)	4	825.8898	0.143984 9	0.477241776	501.4365
			Phi(~1)p(~1)	2	833.9605	8.21471	0.008437286	513.5738
			Phi(~time)p(~1)	17	837.4767	11.7308507	0.001454395	485.6219
		Wave ecotype	Phi(~rel_hab+time)p(~1)	19	752.5437	0	0.87109793	396.9451
			Phi(~time)p(~1)	17	756.9776	4.433951	0.094895760	405.7925
			Phi(~1)p(~1)	2	760.0966	7.552869	0.019951810	440.5431
			Phi(~rel_hab)p(~1)	4	760.7973	8.253624	0.014054500	437.1702
		Hybrid ecotype	Phi(~1)p(~1)	2	860.1521	0	0.705771195	517.238
			Phi(~rel_hab)p(~1)	4	862.7088	2.556685	0.196556255	515.7282
			Phi(~rel_hab+time)p(~1)	19	864.2784	4.12625	0.089672676	485.5252

			Phi(~time)p(~1)	17	869.1118	8.959731	0.007999874	494.7297
CZB	A1	Full	Phi(~ecotype*rel_hab+time)p(~1)	28	3342.896	0	1.00	2082.169
			Phi(~time)p(~1)	20	3362.419	19.52389	0.0000576	2118.304
			Phi(~rel_hab+time)p(~1)	22	3369.123	26.2276	2.02E-06	2120.874
			Phi(~ecotype+time)p(~1)	22	3375.919	33.0238	6.74E-08	2127.67
			Phi(~ecotype*rel_hab)p(~1)	10	3387.677	44.78177	1.89E-10	2164.04
			Phi(~ecotype)p(~1)	4	3402.823	59.92733	9.70E-14	2191.323
			Phi(~rel_hab)p(~1)	4	3408.824	65.92893	4.83E-15	2197.325
			Phi(~1)p(~1)	2	3410.886	67.99014	1.72E-15	2203.407
		Crab release habitat	Phi(~ecotype)p(~1)	4	787.4035	0	0.79	528.1704
			Phi(~ecotype+time)p(~1)	22	790.0856	2.682132	0.21	490.8412
			Phi(~time)p(~1)	20	799.64	12.236555	0.0017424	505.1316
			Phi(~1)p(~1)	2	805.5368	18.133361	0.0000913	550.4114
		Wave release habitat	Phi(~1)p(~1)	2	791.38	0	0.718928163	529.8178

			Phi(~ecotype)p(~1)	4	793.3533	1.973305	0.268032668	527.7107
			Phi(~time)p(~1)	20	800.2486	8.868594	0.008528932	500.1981
			Phi(~ecotype+time)p(~1)	22	801.5228	10.142824	0.004510236	496.9374
		Hybrid release habitat	Phi(~ecotype+time)p(~1)	22	1731.611	0	0.54	991.2571
			Phi(~time)p(~1)	20	1731.96	0.3486687	0.4565260	995.8632
			Phi(~1)p(~1)	2	1759.396	27.784366	5.03E-07	1060.5169
			Phi(~ecotype)p(~1)	4	1759.407	27.795345	5.01E-07	1056.4876
		Crab ecotype	Phi(~time)p(~1)	20	1070.106	0	0.54784509	635.448
			Phi(~rel_hab+time)p(~1)	22	1071.821	1.714502	0.232465338	632.7434
			Phi(~1)p(~1)	2	1072.89	2.784251	0.136164933	676.1955
			Phi(~rel_hab)p(~1)	4	1073.868	3.761703	0.083524599	673.1088
		Wave ecotype	Phi(~rel_hab)p(~1)	4	1093.671	0	0.979605558	704.9419
			Phi(~rel_hab+time)p(~1)	22	1102.078	8.406533	0.014641846	674.8957
			Phi(~1)p(~1)	2	1104.025	10.353391	0.005531474	719.3629

			Phi(~time)p(~1)	20	1110.464	16.792578	0.0002211	687.7256
		Hybrid ecotype	Phi(~rel_hab)p(~1)	4	1219.853	0	0.9106882	781.5966
			Phi(~1)p(~1)	2	1224.508	4.654973	0.08882906	790.3136
			Phi(~rel_hab+time)p(~1)	22	1235.239	15.385337	0.00041541	758.7439
			Phi(~time)p(~1)	20	1238.879	19.025297	6.73099E-05	766.7881
ANG	A2	Full	Phi(~ecotype * rel_hab * weight + time)p(~1)	34	2410.004	0	9.06E-01	2340.029
			Phi(~ecotype * rel_hab + time)p(~1)	25	2415.088	5.084236	7.13E-02	1393.251
			Phi(~ecotype + rel_hab * weight + time)p(~1)	24	2418.019	8.014951	1.65E-02	2369.031
			Phi(~ecotype + time)p(~1)	19	2422.059	12.055447	2.19E-03	1412.67
			Phi(~ecotype + time + weight)p(~1)	20	2423.191	13.187186	1.24E-03	2382.502
			Phi(~ecotype + rel_hab + time)p(~1)	21	2423.225	13.222117	1.22E-03	1409.7
			Phi(~ecotype + rel_hab + weight + time)p(~1)	22	2424.013	14.009249	8.23E-04	2379.182
			Phi(~ecotype * weight + time)p(~1)	22	2427.106	17.101949	1.75E-04	2382.274
			Phi(~ecotype * weight + rel_hab + time)p(~1)	24	2428.02	18.016251	1.11E-04	2379.033
			Phi(~rel_hab * weight + time)p(~1)	22	2430.445	20.441349	3.30E-05	2385.614
			Phi(~time)p(~1)	17	2432.373	22.369215	1.26E-05	1427.106

			Phi(~rel_hab + time)p(~1)	19	2432.798	22.794147	1.02E-05	1423.409
			Phi(~weight + time)p(~1)	18	2434.432	24.427993	4.50E-06	2397.872
			Phi(~rel_hab + time + weight)p(~1)	20	2434.792	24.787786	3.76E-06	2394.103
			Phi(~ecotype * rel_hab * weight)p(~1)	19	2440.07	30.066347	2.68E-07	2401.447
			Phi(~ecotype * rel_hab + weight)p(~1)	11	2447.958	37.95378	5.20E-09	2425.743
			Phi(~ecotype * rel_hab)p(~1)	10	2448.07	38.065704	4.91E-09	1457.124
			Phi(~ecotype + rel_hab * weight)p(~1)	9	2450.099	40.094738	1.78E-09	2431.952
			Phi(~ecotype)p(~1)	4	2453.418	43.413685	3.39E-10	1474.619
			Phi(~ecotype + weight)p(~1)	5	2454.086	44.082219	2.43E-10	2444.038
			Phi(~ecotype + rel_hab)p(~1)	6	2454.542	44.538023	1.93E-10	1471.707
			Phi(~ecotype + rel_hab + weight)p(~1)	7	2454.825	44.821105	1.68E-10	2440.734
			Phi(~ecotype * weight)p(~1)	7	2457.598	47.593605	4.19E-11	2443.507
			Phi(~rel_hab * weight)p(~1)	7	2463.466	53.462205	2.23E-12	2449.375
			Phi(~1)p(~1)	2	2464.255	54.250797	1.50E-12	1489.479
			Phi(~rel_hab)p(~1)	4	2464.782	54.777585	1.15E-12	1485.983
			Phi(~weight)p(~1)	3	2466.231	56.227414	5.59E-13	2460.212
			Phi(~rel_hab + weight)p(~1)	5	2466.627	56.623319	4.59E-13	2456.579

		Crab ecotype	Phi(~rel_hab + time)p(~1)	19	825.7458	0	0.247611868	469.52
			Phi(~rel_hab)p(~1)	4	825.8898	0.143984	0.230412237	501.4365
			Phi(~rel_hab * weight)p(~1)	7	826.0933	0.3474612	0.208123542	811.826
			Phi(~rel_hab + weight)p(~1)	5	826.4923	0.7464759	0.17048111	816.3498
			Phi(~rel_hab + time + weight)p(~1)	20	827.6706	1.9247536	0.094583746	785.6016
			Phi(~rel_hab * weight + time)p(~1)	22	829.3008	3.5549386	0.041862614	782.7958
			Phi(~1)p(~1)	2	833.9605	8.21471	0.004073520	513.5738
			Phi(~weight)p(~1)	3	835.4106	9.6647457	0.001972871	829.3538
			Phi(~time)p(~1)	17	837.4767	11.7308507	0.000702182	485.6219
			Phi(~weight + time)p(~1)	18	840.2406	14.4947487	0.00017631	802.5641
		Wave ecotype	Phi(~rel_hab * weight + time)p(~1)	22	742.8472	0	0.9704679	696.0593
			Phi(~rel_hab * weight)p(~1)	7	750.8362	7.988928	0.017873432	736.5399
			Phi(~rel_hab + time)p(~1)	19	752.5437	9.696454	0.007610671	396.9451
			Phi(~rel_hab + time + weight)p(~1)	20	754.7667	11.919431	0.002504434	712.4653
			Phi(~time)p(~1)	17	756.9776	14.130405	0.000829092	405.7925
			Phi(~weight + time)p(~1)	18	759.1689	16.323691	0.000277186	721.3052

			Phi(~1)p(~1)	2	760.0966	17.249323	0.000174316	440.5431
			Phi(~rel_hab)p(~1)	4	760.7973	17.950078	0.000122792	437.1702
			Phi(~weight)p(~1)	3	761.6105	18.763258	8.17695E-05	755.5477
			Phi(~rel_hab + weight)p(~1)	5	762.2829	19.435696	5.84216E-05	752.125
		Hybrid ecotype	Phi(~1)p(~1)	2	860.1521	0	0.422005793	517.238
			Phi(~weight)p(~1)	3	861.411	1.258866	0.224884332	855.3542
			Phi(~rel_hab)p(~1)	4	862.7088	2.556685	0.117528002	515.7282
			Phi(~rel_hab + time)p(~1)	19	864.2784	4.12625	0.053618494	485.5252
			Phi(~rel_hab * weight + time)p(~1)	22	864.2902	4.138129	0.053300981	817.7853
			Phi(~rel_hab + weight)p(~1)	5	864.306	4.153866	0.052883232	854.1635
			Phi(~rel_hab + time + weight)p(~1)	20	865.2699	5.117774	0.032659384	823.2009
			Phi(~rel_hab * weight)p(~1)	7	865.2918	5.139681	0.032303592	851.0245
			Phi(~weight + time)p(~1)	18	868.6477	8.495619	0.006032791	830.9713
			Phi(~time)p(~1)	17	869.1118	8.959731	0.004783410	494.7297
		Crab release habitat	Phi(~ecotype*weight+time)p(~1)	22	659.963	0	0.763461	611.5438

			Phi(~ecotype*weight)p(~1)	7	664.8908	4.927822	0.06497164	650.4318
			Phi(~ecotype+time+weight)p(~1)	20	665.0074	5.04441	0.06129248	621.371
			Phi(~ecotype+time)p(~1)	19	665.0085	5.045548	0.0612576	399.247
			Phi(~ecotype)p(~1)	4	666.1538	6.190799	0.03455189	433.5062
			Phi(~ecotype+weight)p(~1)	5	668.1753	8.212298	0.01257503	657.9314
			Phi(~weight)p(~1)	3	672.8373	12.87435	0.00122242	666.7406
			Phi(~1)p(~1)	2	674.3888	14.425759	0.000562695	445.8549
			Phi(~weight+time)p(~1)	18	677.9097	17.946748	9.67609E-05	638.9741
			Phi(~time)p(~1)	17	682.7361	22.773141	8.66288E-06	421.6351
		Wave release habitat	Phi(~ecotype*weight+time)p(~1)	22	543.8206	0	0.880418009	496.4132
			Phi(~weight+time)p(~1)	18	549.156	5.335408	0.061111061	510.8836
			Phi(~ecotype+time)p(~1)	19	551.4019	7.581286	0.019880788	298.7933
			Phi(~ecotype+time+weight)p(~1)	20	551.5172	7.696637	0.018766591	508.7079
			Phi(~ecotype*weight)p(~1)	7	551.9811	8.160477	0.014882088	537.6221
			Phi(~1)p(~1)	2	556.3335	12.512907	0.001688673	340.2204

			Phi(~time)p(~1)	17	556.8821	13.061523	0.001283557	308.7804
			Phi(~weight)p(~1)	3	557.4127	13.592102	0.000984468	551.3368
			Phi(~ecotype)p(~1)	4	558.6808	14.860167	0.000522209	338.4785
			Phi(~ecotype+weight)p(~1)	5	558.9234	15.102775	0.000462555	548.7323
		Hybrid release habitat	Phi(~ecotype+time)p(~1)	19	1116.785	0	0.304166371	543.4067
			Phi(~time)p(~1)	17	1117.06	0.275099	0.265078325	547.9131
			Phi(~weight+time)p(~1)	18	1117.162	0.377289 6	0.251874266	1080.1084
			Phi(~ecotype+time+weight)p(~1)	20	1118.722	1.936960 3	0.115479688	1077.4237
			Phi(~ecotype*weight+time)p(~1)	22	1120.827	4.041852 7	0.040311 97	1075.2579
			Phi(~ecotype)p(~1)	4	1123.264	6.478892 3	0.011918939	580.9981
			Phi(~ecotype+weight)p(~1)	5	1124.737	7.952394 9	0.005705196	1114.6468
			Phi(~ecotype*weight)p(~1)	7	1125.395	8.609557 5	0.004107424	1111.2249
			Phi(~weight)p(~1)	3	1128.726	11.940805 1	0.000776602	1122.6897
			Phi(~1)p(~1)	2	1129.305	12.520405 6	0.000581219	591.0819
CZB	A2	Full	Phi(~ecotype*rel_hab+time)p(~1)	28	3342.896	0	0.997754	2082.169

			Phi(~ecotype+rel_hab*weight+time)p(~1)	27	3355.96	13.06451	0.001452452	3300.791
			Phi(~ecotype*weight+time)p(~1)	25	3358.277	15.3816	0.000455987	3307.273
			Phi(~ecotype+rel_hab+time)p(~1)	24	3361.165	18.26946	0.000107612	2108.77
			Phi(~ecotype+rel_hab+weight+time)p(~1)	25	3361.374	18.4788	9.69179E-05	3310.37
			Phi(~time)p(~1)	20	3362.419	19.52389	5.74732E-05	2118.304
			Phi(~ecotype+time+weight)p(~1)	23	3362.641	19.74573	5.1439E-05	3315.79
			Phi(~ecotype*weight+rel_hab+time)p(~1)	27	3364.364	21.46861	2.17358E-05	3309.195
			Phi(~rel_hab+time)p(~1)	22	3369.123	26.2276	2.01268E-06	2120.874
			Phi(~ecotype*rel_hab*weight+time)p(~1)	37	3373.023	30.12727	2.86398E-07	3296.831
			Phi(~ecotype+time)p(~1)	22	3375.919	33.0238	6.72973E-08	2127.67
			Phi(~rel_hab*weight+time)p(~1)	25	3378.395	35.4999	1.95128E-08	3327.392
			Phi(~rel_hab+time+weight)p(~1)	23	3384.659	41.76303	8.5172E-10	3337.807
			Phi(~weight+time)p(~1)	21	3386.373	43.47715	3.61477E-10	3343.661
			Phi(~ecotype*rel_hab)p(~1)	10	3387.677	44.78177	1.88272E-10	2164.04
			Phi(~ecotype*rel_hab+weight)p(~1)	11	3388.725	45.82991	1.11477E-10	3366.524
			Phi(~ecotype*rel_hab*weight)p(~1)	19	3395.493	52.5979	3.78037E-12	3356.909
			Phi(~ecotype+rel_hab)p(~1)	6	3400.8	57.90446	2.66213E-13	2185.267

			Phi(~ecotype+rel_hab+weight)p(~1)	7	3402.105	59.20903	1.38658E-13	3388.019
			Phi(~ecotype)p(~1)	4	3402.823	59.92733	9.68209E-14	2191.323
			Phi(~ecotype+rel_hab*weight)p(~1)	9	3403.222	60.32673	7.92939E-14	3385.085
			Phi(~ecotype+weight)p(~1)	5	3403.891	60.99516	5.67665E-14	3393.845
			Phi(~ecotype*weight)p(~1)	7	3405.825	62.92933	2.1582E-14	3391.74
			Phi(~rel_hab)p(~1)	4	3408.824	65.92893	4.81658E-15	2197.325
			Phi(~rel_hab+weight)p(~1)	5	3410.685	67.78896	1.90037E-15	3400.639
			Phi(~1)p(~1)	2	3410.886	67.99014	1.71851E-15	2203.407
			Phi(~rel_hab*weight)p(~1)	7	3412.273	69.37783	0	3398.188
			Phi(~weight)p(~1)	3	3412.82	69.92456	0	3406.802
		Crab ecotype	Phi(~rel_hab+time+weight)p(~1)	23	1069.952	0	0.256523786	1021.3171
			Phi(~time)p(~1)	20	1070.106	0.1542765	0.24	635.448
			Phi(~weight+time)p(~1)	21	1070.858	0.9065295	0.163033655	1026.6637
			Phi(~rel_hab*weight+time)p(~1)	25	1071.798	1.8460611	0.101920269	1018.6805
			Phi(~rel_hab+time)p(~1)	22	1071.821	1.8687789	0.100769116	632.7434
			Phi(~1)p(~1)	2	1072.89	2.9385279	0.059024786	676.1955

			Phi(~rel_hab)p(~1)	4	1073.868	3.9159793	0.04	673.1088
			Phi(~weight)p(~1)	3	1074.727	4.7754248	0.02	1068.6727
			Phi(~rel_hab+weight)p(~1)	5	1075.80	5.8438549	0.013808633	1065.6585
			Phi(~rel_hab*weight)p(~1)	7	1076.971	7.0186264	0.007674539	1062.7131
		Wave ecotype	Phi(~rel_hab+weight)p(~1)	5	1092.475	0	0.5818069	1082.3302
			Phi(~rel_hab)p(~1)	4	1093.671	1.196358	0.3198844	704.9419
			Phi(~rel_hab*weight)p(~1)	7	1096.321	3.846017	0.08504071	1082.0493
			Phi(~rel_hab+time)p(~1)	22	1102.078	9.602891	0.004781208	674.8957
			Phi(~rel_hab+time+weight)p(~1)	23	1102.493	10.017851	0.00388535	1053.7051
			Phi(~weight)p(~1)	3	1103.592	11.116565	0.002243094	1097.534
			Phi(~1)p(~1)	2	1104.025	11.549749	0.0018062	719.3629
			Phi(~rel_hab*weight+time)p(~1)	25	1106.844	14.369265	0.000441096	1053.5449
			Phi(~time)p(~1)	20	1110.464	17.988936	0.000072199	687.7256
			Phi(~weight+time)p(~1)	21	1111.71	19.234881	3.87238E-05	1067.3884
		Hybrid ecotype	Phi(~rel_hab)p(~1)	4	1219.853	0	0.548184	781.5966

			Phi(~rel_hab+weight)p(~1)	5	1221.76	1.906943	0.2112705	1211.6275
			Phi(~rel_hab*weight)p(~1)	7	1222.242	2.388789	0.166038	1207.9932
			Phi(~1)p(~1)	2	1224.508	4.654973	0.0534701 9	790.3136
			Phi(~weight)p(~1)	3	1226.424	6.571063	0.0205134 2	1220.3715
			Phi(~rel_hab+time)p(~1)	22	1235.239	15.385337	0.0002500 59	758.7439
			Phi(~rel_hab+time+weight)p(~1)	23	1236.419	16.566179	0.0001385 56	1187.8757
			Phi(~weight+time)p(~1)	21	1238.29	18.43666 6	5.4382E-05	1194.1707
			Phi(~time)p(~1)	20	1238.879	19.025297	4.05169E-05	766.7881
			Phi(~rel_hab*weight+time)p(~1)	25	1238.89	19.037159	4.02773E-05	1185.8812
		Crab release habitat	Phi(~ecotype)p(~1)	4	787.4035	0	0.4420741	528.1704
			Phi(~ecotype+weight)p(~1)	5	788.1907	0.787282 5	0.2982214	777.96
			Phi(~ecotype+time)p(~1)	22	790.0856	2.682132 3	0.1156318	490.8412
			Phi(~ecotype+time+weight)p(~1)	23	790.4115	3.007986 8	0.0982469 3	739.8495
			Phi(~ecotype*weight)p(~1)	7	792.2334	4.829891 8	0.0395091 2	777.7993
			Phi(~ecotype*weight+time)p(~1)	25	796.699	9.29555	0.0042363 52	741.2823

			Phi(~weight+time)p(~1)	21	799.5366	12.1331085	0.001025236	753.7497
			Phi(~time)p(~1)	20	799.64	12.2365547	0.000973556	505.1316
			Phi(~1)p(~1)	2	805.5368	18.1333607	5.10371E-05	550.4114
			Phi(~weight)p(~1)	3	806.5674	19.1639463	3.04858E-05	800.4758
		Wave release habitat	Phi(~1)p(~1)	2	791.38	0	0.409414453	529.8178
			Phi(~weight)p(~1)	3	792.0234	0.6434134	0.296788949	785.9549
			Phi(~ecotype)p(~1)	4	7.93E+02	1.9733051	0.152638961	527.7107
			Phi(~ecotype*weight)p(~1)	7	794.9188	3.5388314	0.069777547	780.5951
			Phi(~ecotype+weight)p(~1)	5	795.273	3.8929858	0.058453706	785.1006
			Phi(~time)p(~1)	20	800.2486	8.8685945	0.004857047	500.1981
			Phi(~weight+time)p(~1)	21	800.4759	9.0959045	0.004335235	755.6928
			Phi(~ecotype+time)p(~1)	22	801.5228	10.1428238	0.002568485	496.9374
			Phi(~ecotype+time+weight)p(~1)	23	803.6484	12.2683865	0.000887395	754.303
			Phi(~ecotype*weight+time)p(~1)	25	805.9681	14.5881266	0.000278222	752.0047
		Hybrid release	Phi(~ecotype+time+weight)p(~1)	23	1727.852	0	0.4316386	1680.2216

		habitat						
			Phi(~ecotype*weight+time)p(~1)	25	1728.8	0.9479021	0.2687113	1676.8743
			Phi(~weight+time)p(~1)	21	1729.62	1.767201	0.1783929	1686.2587
			Phi(~ecotype+time)p(~1)	22	1731.611	3.7589016	0.0658999 7	991.2571
			Phi(~time)p(~1)	20	1731.96	4.1075703	0.0553570 2	995.8632
			Phi(~1)p(~1)	2	1759.396	31.5432682	6.10361E-08	1060.5169
			Phi(~ecotype)p(~1)	4	1759.407	31.5542475	6.07019E-08	1056.4876
			Phi(~ecotype*weight)p(~1)	7	1760.674	32.8215924	3.22108E-08	1746.5123
			Phi(~ecotype+weight)p(~1)	5	1760.924	33.0716072	2.84258E-08	1750.8376
			Phi(~weight)p(~1)	3	1761.027	33.1745095	2.70002E-08	1754.9924
ANG	A3	Full	Phi(~ecotype*rel_hab+time)p(~1)	25	2373.389	0	0.9552364	1356.937
			Phi(~ecotype*rel_hab*thick+time)p(~1)	34	2380.16	6.771004	0.0323449 1	2310.145
			Phi(~ecotype+rel_hab+time)p(~1)	21	2383.921	10.53	0.0049327 3	1375.788
			Phi(~ecotype+time)p(~1)	19	2384.989	11.600315	0.0028915 8	1380.994
			Phi(~ecotype+rel_hab+thick+time)p(~1)	22	2385.485	12.096145	0.0022566 6	2340.637
			Phi(~ecotype+time+thick)p(~1)	20	2386.816	13.427092	0.0011599 9	2346.113
			Phi(~ecotype*thick+rel_hab+time)p(~1)	24	2388.664	15.27512	0.0004604 3	2339.657

			Phi(~ecotype+rel_hab*thick+time)p(~1)	24	2389.046	15.65742	0.00038032	2340.039
			Phi(~ecotype*thick+time)p(~1)	22	2390.079	16.69	0.00022698	2345.231
			Phi(~rel_hab+time)p(~1)	19	2393.205	19.816315	4.7539E-05	1389.21
			Phi(~rel_hab+time+thick)p(~1)	20	2394.369	20.979792	2.6571E-05	2353.666
			Phi(~time)p(~1)	17	2394.827	21.437914	2.1131E-05	1394.957
			Phi(~thick+time)p(~1)	18	2396.114	22.724992	1.1103E-05	2359.543
			Phi(~rel_hab*thick+time)p(~1)	22	2398.392	25.002745	3.555E-06	2353.544
			Phi(~ecotype*rel_hab)p(~1)	10	2405.94	32.551036	8.161E-08	1420.398
			Phi(~ecotype*rel_hab+thick)p(~1)	11	2407.975	34.585732	2.9506E-08	2385.756
			Phi(~ecotype*rel_hab*thick)p(~1)	19	2411.531	38.141815	4.9856E-09	2372.895
			Phi(~ecotype+rel_hab)p(~1)	6	2414.764	41.374842	9.901E-10	1437.335
			Phi(~ecotype)p(~1)	4	2415.833	42.443694	5.802E-10	1442.44
			Phi(~ecotype+rel_hab+thick)p(~1)	7	2416.658	43.268578	3.8411E-10	2402.565
			Phi(~ecotype+thick)p(~1)	5	2417.835	44.44645	2.1315E-10	2407.786
			Phi(~ecotype+rel_hab*thick)p(~1)	9	2420.114	46.724917	6.8222E-11	2401.965
			Phi(~ecotype*thick)p(~1)	7	2421.111	47.721978	4.144E-11	2407.018
			Phi(~rel_hab)p(~1)	4	2425.044	51.654794	5.7998E-12	1451.651

			Phi(~1)p(~1)	2	2426.434	53.045156	2.894E-12	1457.065
			Phi(~rel_hab+thick)p(~1)	5	2426.616	53.22685	2.6427E-12	2416.566
			Phi(~thick)p(~1)	3	2428.112	54.723165	1.2506E-12	2422.092
			Phi(~rel_hab*thick)p(~1)	7	2430.512	57.122478	3.7681E-13	2416.419
		Crab ecotype	Phi(~rel_hab+time)p(~1)	19	825.7458	0	0.34953997	469.52
			Phi(~rel_hab)p(~1)	4	825.8898	0.143984 9	0.3252602	501.4365
			Phi(~rel_hab+time+thick)p(~1)	20	827.6249	1.879113 6	0.13660057	785.556
			Phi(~rel_hab+thick)p(~1)	5	827.9147	2.168925 9	0.11817362	817.7722
			Phi(~rel_hab*thick)p(~1)	7	830.2375	4.491701 2	0.03699443	815.9702
			Phi(~rel_hab*thick+time)p(~1)	22	831.0886	5.342778 6	0.02417281	784.5836
			Phi(~1)p(~1)	2	833.9605	8.21471	0.00575036	513.5738
			Phi(~thick)p(~1)	3	835.9888	10.2429857	0.00208574	829.9321
			Phi(~time)p(~1)	17	837.4767	11.7308507	0.00099123	485.6219
			Phi(~thick+time)p(~1)	18	839.1421	13.3962387	0.00043106	801.4656
		Wave ecotype	Phi(~rel_hab*thick+time)p(~1)	22	747.9076	0	0.69444777	701.112
			Phi(~rel_hab+time)p(~1)	19	750.9159	3.008252	0.15431425	396.2197

			Phi(~rel_hab*thick)p(~1)	7	752.7081	4.800432	0.0629852 7	738.411
			Phi(~rel_hab+time+thick)p(~1)	20	752.8652	4.957522	0.0582273 9	710.5575
			Phi(~time)p(~1)	17	755.4302	7.522545	0.0161487 8	405.1486
			Phi(~thick+time)p(~1)	18	757.6216	9.713962	0.0053985 8	719.7527
			Phi(~1)p(~1)	2	758.4211	10.513454	0.0036197	439.7756
			Phi(~rel_hab)p(~1)	4	759.0974	11.189733	0.0025811 9	436.3781
			Phi(~thick)p(~1)	3	760.3951	12.487482	0.0013490 2	754.3321
			Phi(~rel_hab+thick)p(~1)	5	761.1432	13.235551	0.0009280 6	750.9849
		Hybrid ecotype	Phi(~1)p(~1)	2	860.1521	0	0.3197031 7	517.238
			Phi(~thick)p(~1)	3	861.0469	0.8948157	0.2043808 4	854.9902
			Phi(~thick+time)p(~1)	18	861.6652	1.513058 7	0.1500342 5	823.9887
			Phi(~rel_hab)p(~1)	4	862.7088	2.556684 8	0.0890368 7	515.7282
			Phi(~rel_hab*thick)p(~1)	7	863.6703	3.518151 2	0.0550541 6	849.403
			Phi(~rel_hab*thick+time)p(~1)	22	863.7524	3.600268 6	0.0528394 8	817.2474
			Phi(~rel_hab+thick)p(~1)	5	863.87	3.717845 9	0.0498226 7	853.7274
			Phi(~rel_hab+time)p(~1)	19	864.2784	4.12625	0.0406203	485.5252

			Phi(~rel_hab+time+thick)p(~1)	20	864.5828	4.430703	0.0348445	822.5139
			Phi(~time)p(~1)	17	869.1118	8.959730	0.0036238	494.7297
		Crab release habitat	Phi(~ecotype*thick+time)p(~1)	22	664.9928	0	0.2370472	616.5736
			Phi(~ecotype+thick+time)p(~1)	20	664.995	0.002169	0.2367902	621.3587
			Phi(~ecotype+time)p(~1)	19	665.0085	0.015698	0.2351939	399.247
			Phi(~ecotype)p(~1)	4	666.1538	1.160949	0.1326593	433.5062
			Phi(~ecotype*thick)p(~1)	7	666.8851	1.892212	0.0920335	652.426
			Phi(~ecotype+thick)p(~1)	5	668.0222	3.029338	0.0521221	657.7783
			Phi(~thick)p(~1)	3	671.2289	6.236100	0.0104877	665.1322
			Phi(~1)p(~1)	2	674.3888	9.395908	0.0021604	445.8549
			Phi(~thick+time)p(~1)	18	675.1557	10.16285	0.0014723	636.2201
			Phi(~time)p(~1)	17	682.7361	17.74329	3.326E-05	421.6351
		Wave release habitat	Phi(~thick+time)p(~1)	18	546.8557	0	0.6113976	508.5757
			Phi(~ecotype+time)p(~1)	19	549.0434	2.187706	0.2047714	297.3345

			Phi(~ecotype+thick+time)p(~1)	20	550.5467	3.690992	0.0965684	507.7279
			Phi(~ecotype*thick)p(~1)	7	552.2996	5.443899	0.0401972	537.9395
			Phi(~1)p(~1)	2	554.1148	7.259025	0.0162198	338.9096
			Phi(~time)p(~1)	17	554.3166	7.460893	0.0146626	307.1162
			Phi(~thick)p(~1)	3	555.8958	9.04005	0.0066573	549.8196
			Phi(~ecotype)p(~1)	4	556.5973	9.741559	0.0046878	337.3027
			Phi(~ecotype*thick+time)p(~1)	22	557.7374	10.881659	0.0026509	510.3185
			Phi(~ecotype+thick)p(~1)	5	558.1225	11.266743	0.0021866	547.9308
		Hybrid release habitat	Phi(~ecotype*thick+time)p(~1)	22	1115.727	0	0.4021691	1070.1583
			Phi(~ecotype+time)p(~1)	19	1116.785	1.057747	0.2369855	543.4067
			Phi(~time)p(~1)	17	1117.06	1.332846	0.2065308	547.9131
			Phi(~ecotype+thick+time)p(~1)	20	1118.041	2.313908	0.1264591	1076.7429
			Phi(~thick+time)p(~1)	18	1123.105	7.377737	0.0100543	1086.0511
			Phi(~ecotype)p(~1)	4	1123.264	7.53664	0.0092864	580.9981
			Phi(~ecotype+thick)p(~1)	5	1124.599	8.871242	0.0047647	1114.5079

			Phi(~ecotype*thick)p(~1)	7	1125.66	9.932605	0.00280266	1111.4902
			Phi(~thick)p(~1)	3	1129.13	13.403052	0.00049428	1123.0942
			Phi(~1)p(~1)	2	1129.305	13.578153	0.00045285	591.0819
CZB	A3	Full	Phi(~ecotype*rel_hab+time)p(~1)	28	3342.896	0	0.9989742	2082.169
			Phi(~ecotype*rel_hab*thick+time)p(~1)	37	3357.026	14.13057	0.00085337	3280.834
			Phi(~ecotype+rel_hab+time)p(~1)	24	3361.165	18.26946	0.00010774	2108.77
			Phi(~time)p(~1)	20	3362.419	19.52389	5.75435E-05	2118.304
			Phi(~ecotype+rel_hab+thick+time)p(~1)	25	3368.719	25.8237	2.4661E-06	3317.715
			Phi(~rel_hab+time)p(~1)	22	3369.123	26.2276	2.01514E-06	2120.874
			Phi(~ecotype*thick+rel_hab+time)p(~1)	27	3370.601	27.70521	9.62598E-07	3315.431
			Phi(~rel_hab+time+thick)p(~1)	23	3370.786	27.89043	8.77454E-07	3323.935
			Phi(~ecotype+rel_hab*thick+time)p(~1)	27	3372.39	29.49471	3.93423E-07	3317.221
			Phi(~thick+time)p(~1)	21	3372.55	29.65475	3.63168E-07	3329.839
			Phi(~ecotype+time)p(~1)	22	3375.919	33.0238	6.73796E-08	2127.67
			Phi(~ecotype+time+thick)p(~1)	23	3377.962	35.06693	2.42587E-08	3331.111
			Phi(~ecotype*thick+time)p(~1)	25	3379.573	36.6775	1.08427E-08	3328.569
			Phi(~ecotype*rel_hab)p(~1)	10	3387.677	44.78177	1.88502E-10	2164.04

			Phi(~rel_hab*thick+time)p(~1)	25	3387.911	45.0156	1.67703E-10	3336.907
			Phi(~ecotype*rel_hab+thick)p(~1)	11	3389.703	46.80741	6.84626E-11	3367.501
			Phi(~ecotype*rel_hab*thick)p(~1)	19	3399.937	57.0411	4.10428E-13	3361.352
			Phi(~ecotype+rel_hab)p(~1)	6	3400.8	57.90446	2.66539E-13	2185.267
			Phi(~ecotype+rel_hab+thick)p(~1)	7	3402.805	59.90953	9.78057E-14	3388.72
			Phi(~ecotype)p(~1)	4	3402.823	59.92733	9.69393E-14	2191.323
			Phi(~ecotype+thick)p(~1)	5	3404.827	61.93156	3.55866E-14	3394.782
			Phi(~ecotype*thick)p(~1)	7	3405.611	62.71523	2.40499E-14	3391.526
			Phi(~ecotype+rel_hab*thick)p(~1)	9	3406.102	63.20663	1.88108E-14	3387.965
			Phi(~rel_hab)p(~1)	4	3408.824	65.92893	4.82247E-15	2197.325
			Phi(~rel_hab+thick)p(~1)	5	3410.307	67.41116	2.2983E-15	3400.261
			Phi(~1)p(~1)	2	3410.886	67.99014	1.72061E-15	2203.407
			Phi(~thick)p(~1)	3	3412.343	69.44786	0	3406.325
			Phi(~rel_hab*thick)p(~1)	7	3413.646	70.75003	0	3399.56
		Crab ecotype	Phi(~time)p(~1)	20	1070.106	0	0.26915391	635.448
			Phi(~rel_hab+time+thick)p(~1)	23	1070.723	0.6170235	0.19770413	1022.0884

			Phi(~rel_hab*thick+time)p(~1)	25	1071.357	1.2505847	0.1440256	1018.2393
			Phi(~rel_hab+time)p(~1)	22	1071.821	1.7145025	0.1142092	632.7434
			Phi(~thick+time)p(~1)	21	1071.868	1.761753	0.1115426	1027.6732
			Phi(~1)p(~1)	2	1072.89	2.7842514	0.0668972	676.1955
			Phi(~rel_hab)p(~1)	4	1073.868	3.7617029	0.0410352	673.1088
			Phi(~thick)p(~1)	3	1074.602	4.4960484	0.0284247	1068.5476
			Phi(~rel_hab+thick)p(~1)	5	1075.652	5.5458784	0.0168162	1065.5148
			Phi(~rel_hab*thick)p(~1)	7	1076.654	6.5475499	0.0101910	1062.3963
		Wave ecotype	Phi(~rel_hab+time+thick)p(~1)	23	1089.204	0	0.5112555	1040.4166
			Phi(~rel_hab+thick)p(~1)	5	1089.888	0.6838487	0.3631969	1079.7434
			Phi(~rel_hab*thick)p(~1)	7	1093.328	4.1238659	0.0650357	1079.0565
			Phi(~rel_hab)p(~1)	4	1093.671	4.4670068	0.0547822	704.9419
			Phi(~thick+time)p(~1)	21	1099.49	10.2853293	0.0029868	1055.1682
			Phi(~thick)p(~1)	3	1100.986	11.7811135	0.0014138	1094.9279
			Phi(~rel_hab+time)p(~1)	22	1102.078	12.8735396	0.0008188	674.8957
			Phi(~1)p(~1)	2	1104.025	14.8203982	0.0003093	719.3629

			Phi(~rel_hab*thick+time)p(~1)	25	1105.016	15.8115136	0.000188457	1051.7165
			Phi(~time)p(~1)	20	1110.464	21.2595844	1.23645E-05	687.7256
		Hybrid ecotype	Phi(~rel_hab)p(~1)	4	1219.853	0	0.4671176	781.5966
			Phi(~rel_hab*thick)p(~1)	7	1221.184	1.330289	0.2401916	1206.9347
			Phi(~rel_hab+thick)p(~1)	5	1221.341	1.488043	0.2219738	1211.2086
			Phi(~1)p(~1)	2	1224.508	4.654973	0.04556293	790.3136
			Phi(~thick)p(~1)	3	1225.733	5.879263	0.02470361	1219.6797
			Phi(~rel_hab+time)p(~1)	22	1235.239	15.385337	0.00021308	758.7439
			Phi(~rel_hab+time+thick)p(~1)	23	1237.118	17.264479	8.32707E-05	1188.574
			Phi(~rel_hab*thick+time)p(~1)	25	1237.644	17.791159	6.39919E-05	1184.6352
			Phi(~thick+time)p(~1)	21	1237.926	18.072666	5.559E-05	1193.8067
			Phi(~time)p(~1)	20	1238.879	19.025297	3.45252E-05	766.7881
		Crab release habitat	Phi(~ecotype+time+thick)p(~1)	23	787.0527	0	0.3493263	736.4907
			Phi(~ecotype)p(~1)	4	787.4035	0.3507432	0.2931355	528.1704
			Phi(~ecotype+thick)p(~1)	5	788.7194	1.6666858	0.1518151	778.4886

			Phi(~ecotype+time)p(~1)	22	790.0856	3.0328756	0.07667446	490.8412
			Phi(~ecotype*thick+time)p(~1)	25	790.1122	3.0594732	0.07566153	734.6955
			Phi(~ecotype*thick)p(~1)	7	791.1784	4.1256951	0.04439643	776.7443
			Phi(~thick+time)p(~1)	21	794.5451	7.4923818	0.00824672	748.7582
			Phi(~time)p(~1)	20	799.64	12.587298	0.00064555 7	505.1316
			Phi(~thick)p(~1)	3	804.2459	17.1931296	6.45343E-05	798.1543
			Phi(~1)p(~1)	2	805.5368	18.4841039	3.38422E-05	550.4114
		Wave release habitat	Phi(~ecotype+time+thick)p(~1)	23	791.2717	0	0.25805608 1	741.9262
			Phi(~1)p(~1)	2	791.38	0.1083235	0.24445107 4	529.8178
			Phi(~ecotype*thick)p(~1)	7	791.5425	0.2707649	0.22538149 3	777.2188
			Phi(~thick)p(~1)	3	792.48	1.2083369	0.14103505 6	786.4115
			Phi(~ecotype)p(~1)	4	793.3533	2.0816286	0.09113688 5	527.7107
			Phi(~ecotype+thick)p(~1)	5	795.4089	4.1372492	0.03260782 5	785.2365
			Phi(~time)p(~1)	20	800.2486	8.976918	0.00290002 1	500.1981
			Phi(~thick+time)p(~1)	21	800.7854	9.513678	0.00221740 6	756.0022

			Phi(~ecotype+time)p(~1)	22	801.5228	10.251173	0.001533578	496.9374
			Phi(~ecotype*thick+time)p(~1)	25	803.1477	11.8759701	0.000680581	749.1843
		Hybrid release habitat	Phi(~ecotype+time+thick)p(~1)	23	1727.753	0	0.6119028	1680.1227
			Phi(~ecotype*thick+time)p(~1)	25	1730.004	2.250402	0.1986158	1678.0779
			Phi(~ecotype+time)p(~1)	22	1731.611	3.857802	0.08891427	991.2571
			Phi(~time)p(~1)	20	1731.96	4.20647	0.0746894	995.8632
			Phi(~thick+time)p(~1)	21	1734.08	6.326401	0.02587748	1690.719
			Phi(~1)p(~1)	2	1759.396	31.642168	8.23518E-08	1060.5169
			Phi(~ecotype)p(~1)	4	1759.407	31.653147	8.19009E-08	1056.4876
			Phi(~ecotype+thick)p(~1)	5	1760.442	32.688307	4.88098E-08	1750.3554
			Phi(~thick)p(~1)	3	1761.178	33.42491	3.37719E-08	1755.1439
			Phi(~ecotype*thick)p(~1)	7	1761.305	33.551792	3.16959E-08	1747.1436
ANG	A4	Full	Phi(~ecotype*rel_hab*PC1+time)p(~1)	34	2414.007	0	0.5876471	1373.266
			Phi(~ecotype*rel_hab+time)p(~1)	25	2415.088	1.080836	0.3423071	1393.251
			Phi(~ecotype+rel_hab*PC1+time)p(~1)	24	2419.071	5.063751	0.04672369	1399.317

			Phi(~ecotype+time)p(~1)	19	2422.059	8.052047	0.01048665	1412.67
			Phi(~ecotype+rel_hab+time)p(~1)	21	2423.225	9.217717	0.00585484	1409.7
			Phi(~ecotype+time+PC1)p(~1)	20	2424.125	10.118086	0.00373252	1412.67
			Phi(~ecotype+rel_hab+PC1+time)p(~1)	22	2425.298	11.290449	0.00207695	1409.7
			Phi(~ecotype*PC1+time)p(~1)	22	2427.797	13.789849	0.00059524	1412.199
			Phi(~rel_hab*PC1+time)p(~1)	22	2428.854	14.846749	0.0003509	1413.256
			Phi(~ecotype*PC1+rel_hab+time)p(~1)	24	2431.986	17.978351	7.3311E-05	1412.232
			Phi(~time)p(~1)	17	2432.373	18.365815	6.0399E-05	1427.106
			Phi(~rel_hab+time)p(~1)	19	2432.798	18.790747	4.8838E-05	1423.409
			Phi(~PC1+time)p(~1)	18	2434.432	20.425093	2.1571E-05	1427.106
			Phi(~rel_hab+time+PC1)p(~1)	20	2434.864	20.856886	1.7382E-05	1423.409
			Phi(~ecotype+rel_hab*PC1)p(~1)	9	2439.772	25.764238	1.4945E-06	1450.859
			Phi(~ecotype*rel_hab*PC1)p(~1)	19	2439.94	25.932147	1.3741E-06	1430.55
			Phi(~ecotype*rel_hab+PC1)p(~1)	11	2442.109	28.10208	4.6433E-07	1449.128
			Phi(~ecotype+PC1)p(~1)	5	2447.109	33.101119	3.8133E-08	1466.293
			Phi(~ecotype*rel_hab)p(~1)	10	2448.07	34.062304	2.3582E-08	1457.124
			Phi(~ecotype+rel_hab+PC1)p(~1)	7	2448.762	34.754905	1.668E-08	1463.905

			Phi(~ecotype*PC1)p(~1)	7	2450.347	36.339505	7.5525E-09	1465.49
			Phi(~rel_hab*PC1)p(~1)	7	2450.839	36.831105	5.9067E-09	1465.981
			Phi(~ecotype)p(~1)	4	2453.418	39.410285	1.6266E-09	1474.619
			Phi(~ecotype+rel_hab)p(~1)	6	2454.542	40.534623	9.2712E-10	1471.707
			Phi(~PC1)p(~1)	3	2457.846	43.838114	1.7774E-10	1481.06
			Phi(~rel_hab+PC1)p(~1)	5	2458.984	44.976319	1.0061E-10	1478.169
			Phi(~1)p(~1)	2	2464.255	50.247397	7.2116E-12	1489.479
			Phi(~rel_hab)p(~1)	4	2464.782	50.774185	5.5417E-12	1485.983
		Crab ecotype	Phi(~rel_hab+PC1)p(~1)	5	824.8755	0	0.34445444	498.3745
			Phi(~rel_hab+time)p(~1)	19	825.7458	0.8703541	0.22291373	469.52
			Phi(~rel_hab)p(~1)	4	825.8898	1.0143389	0.20742968	501.4365
			Phi(~rel_hab*PC1)p(~1)	7	827.4129	2.5374153	0.0968588	496.7871
			Phi(~rel_hab+time+PC1)p(~1)	20	827.9474	3.0719877	0.07414095	469.52
			Phi(~rel_hab*PC1+time)p(~1)	22	829.1045	4.2290227	0.04157298	466.2411
			Phi(~PC1)p(~1)	3	832.3773	7.5018198	0.00809342	509.9621
			Phi(~1)p(~1)	2	833.9605	9.0850641	0.00366721	513.5738

			Phi(~time)p(~1)	17	837.4767	12.6012047	0.0006321 4	485.6219
			Phi(~PC1+time)p(~1)	18	839.4417	14.5662528	0.0002366 5	485.4068
		Wave ecotype	Phi(~rel_hab*PC1+time)p(~1)	22	743.8449	0	0.9221262 3	381.5349
			Phi(~rel_hab*PC1)p(~1)	7	749.3378	5.492918	0.0591586 8	419.5195
			Phi(~rel_hab+time)p(~1)	19	752.5437	8.698764	0.0119090 7	396.9451
			Phi(~rel_hab+time+PC1)p(~1)	20	754.7685	10.923631	0.0039152	396.9451
			Phi(~time)p(~1)	17	756.9776	13.132715	0.0012973 5	405.7925
			Phi(~PC1+time)p(~1)	18	759.1784	15.333431	0.0004317	405.7925
			Phi(~PC1)p(~1)	3	759.5758	15.730888	0.0003538 9	437.9909
			Phi(~rel_hab+PC1)p(~1)	5	759.6386	15.793636	0.0003429 6	433.9586
			Phi(~1)p(~1)	2	760.0966	16.251633	0.0002727 7	440.5431
			Phi(~rel_hab)p(~1)	4	760.7973	16.952388	0.0001921 4	437.1702
		Hybrid ecotype	Phi(~1)p(~1)	2	860.1521	0	0.4039986	517.238
			Phi(~PC1)p(~1)	3	860.995	0.8428857	0.2650632 7	516.0524
			Phi(~rel_hab)p(~1)	4	862.7088	2.5566848	0.1125130 2	515.7282

			Phi(~rel_hab+PC1)p(~1)	5	863.7284	3.5762859	0.0675770 5	514.7
			Phi(~PC1+time)p(~1)	18	864.0847	3.9326087	0.0565489 7	487.5224
			Phi(~rel_hab+time)p(~1)	19	864.2784	4.12625	0.0513305 7	485.5252
			Phi(~rel_hab*PC1)p(~1)	7	866.3343	6.1821912	0.0183626	513.1812
			Phi(~rel_hab+time+PC1)p(~1)	20	866.48	6.3278736	0.0170726	485.5252
			Phi(~time)p(~1)	17	869.1118	8.9597306	0.0045793	494.7297
			Phi(~rel_hab*PC1+time)p(~1)	22	869.9886	9.8364886	0.0029540 2	484.5978
		Crab release habitat	Phi(~ecotype+time)p(~1)	19	665.0085	0	0.3335201	399.247
			Phi(~ecotype+time+PC1)p(~1)	20	665.0169	0.0083615 7	0.3321286	396.8949
			Phi(~ecotype)p(~1)	4	666.1538	1.1452512 5	0.1881195	433.5062
			Phi(~ecotype+PC1)p(~1)	5	667.8744	2.8658803 7	0.0795800 1	433.1449
			Phi(~ecotype*PC1+time)p(~1)	22	668.7133	3.7047619 1	0.0523170 4	395.8084
			Phi(~ecotype*PC1)p(~1)	7	672.1216	7.1130143 2	0.0095181 1	433.1769
			Phi(~1)p(~1)	2	674.3888	9.3802107	0.0030636 2	445.8549
			Phi(~PC1)p(~1)	3	675.5788	10.570262 1	0.0016897 4	444.9964

			Phi(~time)p(~1)	17	682.7361	17.7275925	4.7165E-05	421.6351
			Phi(~PC1+time)p(~1)	18	684.8815	19.8729602	1.6135E-05	421.4602
		Wave release habitat	Phi(~PC1+time)p(~1)	18	550.0957	0	0.5380957	299.7481
			Phi(~ecotype+time)p(~1)	19	551.4019	1.306158	0.2800470	298.7933
			Phi(~ecotype+time+PC1)p(~1)	20	553.6779	3.582189	0.0897422	298.7933
			Phi(~1)p(~1)	2	556.3335	6.23778	0.0237871	340.2204
			Phi(~PC1)p(~1)	3	556.6025	6.506804	0.0207933	338.4513
			Phi(~time)p(~1)	17	556.8821	6.786395	0.0180805	308.7804
			Phi(~ecotype*PC1+time)p(~1)	22	557.4048	7.309062	0.0139224	297.9221
			Phi(~ecotype)p(~1)	4	558.6808	8.585039	0.0073560	338.4785
			Phi(~ecotype+PC1)p(~1)	5	558.8846	8.788908	0.0066431	336.6183
			Phi(~ecotype*PC1)p(~1)	7	561.8184	11.722669	0.0015322	335.3842
		Hybrid release habitat	Phi(~ecotype+time+PC1)p(~1)	20	1113.823	0	0.5495146	538.3192
			Phi(~ecotype+time)p(~1)	19	1116.785	2.96204	0.1249627	543.4067

			Phi(~time)p(~1)	17	1117.06	3.237139	0.10890393	547.9131
			Phi(~ecotype*PC1+time)p(~1)	22	1117.497	3.674392	0.08751754	537.7229
			Phi(~ecotype+PC1)p(~1)	5	1118.056	4.233335	0.06617933	573.7601
			Phi(~PC1+time)p(~1)	18	1119.04	5.217429	0.04046028	547.7809
			Phi(~ecotype*PC1)p(~1)	7	1121.297	7.474397	0.01308985	572.9222
			Phi(~ecotype)p(~1)	4	1123.264	9.440932	0.00489674	580.9981
			Phi(~PC1)p(~1)	3	1123.554	9.730745	0.00423618	583.3121
			Phi(~1)p(~1)	2	1129.305	15.482445	0.00023879	591.0819
CZB	A4	Full	Phi(~ecotype*rel_hab+time)p(~1)	28	3342.896	0	0.9656039	2082.169
			Phi(~ecotype*rel_hab*PC1+time)p(~1)	37	3349.963	7.067572	0.02819001	2070.302
			Phi(~ecotype+time)p(~1)	27	3355.435	12.539108	0.00182796	2096.796
			Phi(~ecotype+rel_hab+PC1+time)p(~1)	25	3355.521	12.625295	0.00175086	2101.048
			Phi(~ecotype+time+PC1)p(~1)	23	3356.26	13.364229	0.00121002	2105.939
			Phi(~ecotype*PC1+time)p(~1)	25	3356.276	13.380895	0.00119998	2101.803
			Phi(~ecotype+rel_hab+time)p(~1)	24	3361.165	18.26946	0.00010414	2108.77
			Phi(~time)p(~1)	20	3362.419	19.523888	5.5621E-05	2118.304
			Phi(~rel_hab+time+PC1)p(~1)	23	3363.926	21.030729	2.6184E-05	2113.606

			Phi(~PC1+time)p(~1)	21	3364.485	21.58905	1.9806E-05	2118.304
			Phi(~rel_hab*PC1+time)p(~1)	25	3366.122	23.226695	8.7335E-06	2111.649
			Phi(~rel_hab+time)p(~1)	22	3369.123	26.227595	1.9478E-06	2120.874
			Phi(~ecotype+rel_hab*PC1+time)p(~1)	27	3371.065	28.169808	7.3757E-07	2112.427
			Phi(~ecotype+time)p(~1)	22	3375.919	33.023795	6.5129E-08	2127.67
			Phi(~ecotype*rel_hab*PC1)p(~1)	19	3377.998	35.1027	2.3033E-08	2135.945
			Phi(~ecotype*rel_hab)p(~1)	10	3387.677	44.781773	1.8221E-10	2164.04
			Phi(~ecotype*rel_hab+PC1)p(~1)	11	3389.307	46.411015	8.0682E-11	2163.636
			Phi(~ecotype+rel_hab*PC1)p(~1)	9	3395.07	52.174534	4.5211E-12	2173.463
			Phi(~ecotype+rel_hab)p(~1)	6	3400.8	57.904461	2.5764E-13	2185.267
			Phi(~ecotype*PC1)p(~1)	7	3401.884	58.988135	1.4986E-13	2184.329
			Phi(~ecotype+rel_hab+PC1)p(~1)	7	3401.939	59.043035	1.458E-13	2184.384
			Phi(~ecotype)p(~1)	4	3402.823	59.927329	9.3701E-14	2191.323
			Phi(~rel_hab*PC1)p(~1)	7	3403.633	60.737635	6.2487E-14	2186.079
			Phi(~ecotype+PC1)p(~1)	5	3404.194	61.298861	4.7198E-14	2190.68
			Phi(~rel_hab)p(~1)	4	3408.824	65.928929	4.6614E-15	2197.325
			Phi(~rel_hab+PC1)p(~1)	5	3410.009	67.113361	2.5782E-15	2196.494

			Phi(~1)p(~1)	2	3410.886	67.990139	1.6631E-15	2203.407
			Phi(~PC1)p(~1)	3	3412.315	69.419257	0	2202.827
		Crab ecotype	Phi(~rel_hab*PC1+time)p(~1)	25	1068.428	0	0.3337355	622.6424
			Phi(~time)p(~1)	20	1070.106	1.678615	0.1441769	635.448
			Phi(~rel_hab*PC1)p(~1)	7	1070.193	1.765865	0.1380224	663.2682
			Phi(~rel_hab+time+PC1)p(~1)	23	1070.906	2.478839	0.0966339	629.6039
			Phi(~PC1)p(~1)	3	1071.639	3.211564	0.0669915	672.9168
			Phi(~rel_hab+time)p(~1)	22	1071.821	3.393118	0.0611781	632.7434
			Phi(~PC1+time)p(~1)	21	1071.933	3.505668	0.0578304	635.0707
			Phi(~rel_hab+PC1)p(~1)	5	1072.497	4.069894	0.0436150	669.6925
			Phi(~1)p(~1)	2	1072.89	4.462867	0.0358346	676.1955
			Phi(~rel_hab)p(~1)	4	1073.868	5.440318	0.0219812	673.1088
		Wave ecotype	Phi(~rel_hab*PC1+time)p(~1)	25	1079.128	0	0.9902177	645.1954
			Phi(~rel_hab*PC1)p(~1)	7	1088.788	9.660052	0.0079081	693.8831
			Phi(~rel_hab+time+PC1)p(~1)	23	1093.503	14.374686	0.0007487	664.0817
			Phi(~rel_hab)p(~1)	4	1093.671	14.543393	0.0006881	704.9419

			Phi(~rel_hab+PC1)p(~1)	5	1094.654	15.525735	0.0004210 8	703.8757
			Phi(~rel_hab+time)p(~1)	22	1102.078	22.949926	1.0285E-05	674.8957
			Phi(~1)p(~1)	2	1104.025	24.896785	3.8856E-06	719.3629
			Phi(~PC1)p(~1)	3	1105.547	26.4185	1.8156E-06	718.8557
			Phi(~time)p(~1)	20	1110.464	31.335971	1.5531E-07	687.7256
			Phi(~PC1+time)p(~1)	21	1113.771	34.643016	2.9723E-08	688.8163
		Hybrid ecotype	Phi(~rel_hab)p(~1)	4	1219.853	0	0.4916275	781.5966
			Phi(~rel_hab*PC1)p(~1)	7	1221.351	1.498089	0.2324504	776.9342
			Phi(~rel_hab+PC1)p(~1)	5	1221.581	1.727443	0.2072653	781.2796
			Phi(~1)p(~1)	2	1224.508	4.654973	0.0479536 3	790.3136
			Phi(~PC1)p(~1)	3	1226.237	6.383663	0.0202041 8	790.0158
			Phi(~rel_hab+time)p(~1)	22	1235.239	15.385337	0.0002242 6	758.7439
			Phi(~rel_hab+time+PC1)p(~1)	23	1236.608	16.754779	0.0001130 8	757.896
			Phi(~rel_hab*PC1+time)p(~1)	25	1237.35	17.497059	7.8019E-05	754.1728
			Phi(~PC1+time)p(~1)	21	1238.352	18.498966	4.7276E-05	764.0647
			Phi(~time)p(~1)	20	1238.879	19.025297	3.6337E-05	766.7881

		Crab release habitat	Phi(~ecotype*PC1)p(~1)	7	785.2696	0	0.4904314	519.7557
			Phi(~ecotype*PC1+time)p(~1)	25	786.93	1.660378	0.2138118	480.4336
			Phi(~ecotype)p(~1)	4	787.4035	2.133838	0.1687412	528.1704
			Phi(~ecotype+PC1)p(~1)	5	789.191	3.921411	0.06903269	527.8805
			Phi(~ecotype+time)p(~1)	22	790.0856	4.815971	0.04413708	490.8412
			Phi(~ecotype+time+PC1)p(~1)	23	792.483	7.213355	0.01331123	490.8412
			Phi(~time)p(~1)	20	799.64	14.370393	0.00037161	505.1316
			Phi(~PC1+time)p(~1)	21	801.9983	16.728707	0.00011428	505.1317
			Phi(~PC1)p(~1)	3	804.7334	19.463745	2.9113E-05	547.562
			Phi(~1)p(~1)	2	805.5368	20.267199	1.9481E-05	550.4114
		Wave release habitat	Phi(~1)p(~1)	2	791.38	0	0.50814197	529.8178
			Phi(~PC1)p(~1)	3	793.2084	1.828383	0.20368388	529.6118
			Phi(~ecotype)p(~1)	4	793.3533	1.973305	0.18944681	527.7107
			Phi(~ecotype+PC1)p(~1)	5	795.2045	3.824476	0.07507766	527.5041

			Phi(~ecotype*PC1)p(~1)	7	798.969	7.589001	0.0114302 1	527.1173
			Phi(~time)p(~1)	20	800.2486	8.868594	0.0060282 9	500.1981
			Phi(~ecotype+time)p(~1)	22	801.5228	10.142824	0.0031878 6	496.9374
			Phi(~PC1+time)p(~1)	21	802.4904	11.110414	0.0019651 2	500.1793
			Phi(~ecotype+time+PC1)p(~1)	23	803.9728	12.592797	0.0009364 7	497.0994
			Phi(~ecotype*PC1+time)p(~1)	25	808.4123	17.032307	0.0001017 3	496.9209
		Hybrid release habitat	Phi(~ecotype*PC1+time)p(~1)	25	1727.416	0	0.529948	980.6282
			Phi(~PC1+time)p(~1)	21	1729.544	2.128199	0.1828525	991.3214
			Phi(~ecotype+time+PC1)p(~1)	23	1729.719	2.303198	0.1675329	987.2265
			Phi(~ecotype+time)p(~1)	22	1731.611	4.195699	0.0650352 4	991.2571
			Phi(~time)p(~1)	20	1731.96	4.544368	0.0546306 4	995.8632
			Phi(~ecotype*PC1)p(~1)	7	1755.153	27.73739	5.025E-07	1046.1298
			Phi(~1)p(~1)	2	1759.396	31.980066	6.0235E-08	1060.5169
			Phi(~ecotype)p(~1)	4	1759.407	31.991045	5.9905E-08	1056.4876
			Phi(~ecotype+PC1)p(~1)	5	1760.782	33.366105	3.0121E-08	1055.8338

			Phi(~PC1)p(~1)	3	1761.046	33.630007	2.6398E-08	1060.1496
ANG	A5*	Full	Phi(~ecotype * rel_hab * PC1 + time)p(~1)	34	2372.86	0	0.2420572	1337.485
			Phi(~ecotype * rel_hab + time)p(~1)	25	2373.389	0.5290957	0.1857917	1356.937
			Phi(~ecotype * rel_hab * weight + time)p(~1)	34	2374.313	1.4532	0.1170468	2304.298
			Phi(~ecotype * rel_hab + thick + time)p(~1)	26	2375.235	2.3754829	0.0738054	2322.055
			Phi(~ecotype * rel_hab + PC1 + time)p(~1)	26	2375.477	2.6174829	0.0653941	1356.937
			Phi(~ecotype + rel_hab * PC1 + time)p(~1)	24	2375.775	2.9155154	0.0563406	1361.408
			Phi(~ecotype * rel_hab * weight + thick + time)p(~1)	35	2376.174	3.3136519	0.0461708	2304.038
			Phi(~ecotype * rel_hab + thick * PC1 + time)p(~1)	28	2376.283	3.4231137	0.0437117	2318.915
			Phi(~ecotype * rel_hab * weight + PC1 + time)p(~1)	35	2376.464	3.6044519	0.0399228	2304.329
			Phi(~ecotype * rel_hab + thick + PC1 + time)p(~1)	27	2377.327	4.467486	0.0259307	2322.055
			Phi(~ecotype * rel_hab + weight + time)p(~1)	26	2377.662	4.8024829	0.0219316	2324.482
			Phi(~ecotype + rel_hab * weight * PC1 + time)p(~1)	30	2378.781	5.9205789	0.0125395	2317.211
			Phi(~ecotype * rel_hab + weight * thick + time)p(~1)	28	2379.225	6.3646137	0.0100429	2321.856
			Phi(~ecotype + rel_hab * thick * PC1 + time)p(~1)	30	2379.289	6.4292789	0.0097233	2317.72
			Phi(~ecotype * rel_hab + weight + thick + time)p(~1)	27	2379.358	6.497886	0.0093955	2324.085
			Phi(~ecotype * rel_hab + weight + PC1 + time)p(~1)	27	2379.754	6.894286	0.0077062	2324.481

			Phi(~ecotype * rel_hab + weight * PC1 + time)p(~1)	28	2379.795	6.9350137	0.0075509	2322.427
			Phi(~ecotype * rel_hab * thick + time)p(~1)	34	2380.16	7.3001	0.0062910 3	2310.145
			Phi(~ecotype * rel_hab * weight * PC1 + time)p(~1)	52	2381.109	8.2487256	0.0039149 8	2272.369
			Phi(~ecotype + rel_hab * weight + time)p(~1)	24	2381.137	8.2771154	0.0038598	2332.13
CZB	A5*	Full	Phi(~ecotype * rel_hab + time)p(~1)	28	3342.896	0	0.2454894	2082.169
			Phi(~ecotype * rel_hab + thick * PC1 + time)p(~1)	31	3343.265	0.3698117	0.2040465	3279.726
			Phi(~ecotype * rel_hab + weight + thick * time)p(~1)	48	3343.915	1.0198473	0.1474266	3244.217
			Phi(~ecotype * rel_hab + weight * PC1 + time)p(~1)	31	3344.956	2.0609117	0.0876014 8	3281.417
			Phi(~ecotype * rel_hab + PC1 + time)p(~1)	29	3344.986	2.0909265	0.0862966 3	2082.169
			Phi(~ecotype * rel_hab + weight + thick + time)p(~1)	30	3345.283	2.3876945	0.0743963 2	3283.841
			Phi(~ecotype * rel_hab + weight + PC1 + time)p(~1)	30	3345.703	2.8074945	0.0603105 2	3284.261
			Phi(~ecotype * rel_hab + thick + PC1 + time)p(~1)	30	3347.058	4.1623945	0.0306323 5	3285.616
			Phi(~ecotype * rel_hab + weight * thick + time)p(~1)	31	3347.142	4.2460117	0.0293780 5	3283.602
			Phi(~ecotype * rel_hab + weight + time)p(~1)	29	3348.093	5.1971265	0.0182595 9	3288.745
			Phi(~ecotype * rel_hab * PC1 + time)p(~1)	37	3349.963	7.0675722	0.0071668 6	2070.302
			Phi(~ecotype + rel_hab + thick * PC1 + time)p(~1)	27	3353.633	10.7379076	0.0011437 4	3298.464
			Phi(~ecotype * rel_hab * weight + thick * time)p(~1)	56	3353.703	10.807867	0.0011044 2	3236.653

			Phi(~ecotype + weight + thick * PC1 + time)p(~1)	26	3354.048	11.1520418	0.00092982	3300.963
			Phi(~ecotype * rel_hab * weight + thick + time)p(~1)	38	3354.838	11.9423465	0.0006263	3276.526
			Phi(~ecotype + rel_hab + weight * PC1 + time)p(~1)	27	3355.499	12.6038076	0.00044994	3300.33
			Phi(~ecotype + rel_hab + PC1 + time)p(~1)	25	3355.521	12.6252951	0.00044513	2101.048
			Phi(~ecotype + rel_hab * weight + time)p(~1)	27	3355.96	13.0645076	0.00035736	3300.791
			Phi(~ecotype * rel_hab * weight + PC1 + time)p(~1)	38	3355.975	13.0796465	0.00035467	3277.663
			Phi(~ecotype + weight * PC1 + time)p(~1)	25	3356.121	13.2254951	0.00032973	3305.117
ANG	B	Full	Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab + time)	45	2258.817	0	0.9210544	1194.583
			Phi(~ecotype * rel_hab + time)p(~ecotype + rel_hab + time)	41	2263.732	4.915655	0.07886139	1208.091
			Phi(~ecotype * rel_hab + time)p(~rel_hab + time)	39	2277.416	18.599763	8.42167E-05	1226.05
			Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab)	33	2294.178	35.36126	1.93057E-08	1255.551
			Phi(~ecotype * rel_hab + time)p(~ecotype + rel_hab)	29	2300.116	41.29928	9.91427E-10	1269.912
			Phi(~ecotype * rel_hab + time)p(~rel_hab)	27	2306.353	47.536588	4.38376E-11	1280.339
			Phi(~ecotype * rel_hab + time)p(~time)	37	2358.125	99.308498	0	1311.019
			Phi(~ecotype * rel_hab + time)p(~ecotype + time)	39	2360.926	102.109563	0	1309.56
			Phi(~ecotype * rel_hab + time)p(~1)	25	2415.088	156.271504	0	1393.251
			Phi(~ecotype * rel_hab + time)p(~ecotype)	27	2420.707	161.890388	0	1394.693

CZB	B	Full	Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab + time)	52	3090.252	0	0.9995144	1778.435
			Phi(~ecotype * rel_hab + time)p(~ecotype + rel_hab + time)	48	3105.523	15.27101	0.0004827 6	1802.355
			Phi(~ecotype * rel_hab + time)p(~rel_hab + time)	46	3115.773	25.52103	2.8706E-06	1816.909
			Phi(~ecotype * rel_hab + time)p(~ecotype + time)	46	3131.419	41.16743	1.1492E-09	1832.556
			Phi(~ecotype * rel_hab + time)p(~time)	44	3135.34	45.08875	1.6177E-10	1840.768
			Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab)	36	3276.403	186.1513 6	0	1998.859
			Phi(~ecotype * rel_hab + time)p(~ecotype + rel_hab)	32	3285.706	195.4539 5	0	2016.597
			Phi(~ecotype * rel_hab + time)p(~rel_hab)	30	3289.51	199.2587 6	0	2024.599
			Phi(~ecotype * rel_hab + time)p(~1)	28	3342.896	252.6439 6	0	2082.169
			Phi(~ecotype * rel_hab + time)p(~ecotype)	30	3343.707	253.4553 6	0	2078.796
ANG	C**	Full	Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab + time)	45	2258.817	0	0.735063	1194.583
			Phi(~ecotype + rel_hab + time)p(~ecotype + rel_hab + time)	37	2262.639	3.822298	0.1087234	1215.533
			Phi(~ecotype + rel_hab + time)p(~ecotype * rel_hab + time)	41	2263.09	4.273255	0.0867758 9	1207.449
			Phi(~ecotype * rel_hab + time)p(~ecotype + rel_hab + time)	41	2263.732	4.915655	0.0629366 6	1208.091
			Phi(~rel_hab + time)p(~ecotype * rel_hab + time)	39	2269.15	10.33366 3	0.0041917 76	1217.784
			Phi(~rel_hab + time)p(~ecotype + rel_hab + time)	35	2270.431	11.61438 7	0.0022094 93	1227.572
			Phi(~ecotype * rel_hab + time)p(~rel_hab + time)	39	2277.416	18.59976 3	6.72105E-05	1226.05

			Phi(~ecotype + rel_hab + time)p(~rel_hab + time)	35	2279.308	20.491687	2.60983E-05	1236.449
			Phi(~ecotype + time)p(~ecotype * rel_hab + time)	39	2283.334	24.517763	3.48626E-06	1231.968
			Phi(~ecotype + time)p(~ecotype + rel_hab + time)	35	2284.244	25.427387	2.21227E-06	1241.385
CZB	C**	Full	Phi(~ecotype + rel_hab)p(~ecotype * rel_hab + time)	30	3088.421	0	0.652753	1823.51
			Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab + time)	52	3090.252	1.830743	0.2613414	1778.435
			Phi(~rel_hab)p(~ecotype * rel_hab + time)	28	3094.295	5.873805	0.0346153 1	1833.568
			Phi(~ecotype)p(~ecotype * rel_hab + time)	28	3094.788	6.367605	0.0270421 3	1834.062
			Phi(~ecotype + rel_hab + time)p(~ecotype * rel_hab + time)	48	3095.38	6.958653	0.0201231 8	1792.212
			Phi(~ecotype + time)p(~ecotype * rel_hab + time)	46	3099.829	11.407674	0.0021757 3	1800.965
			Phi(~1)p(~ecotype * rel_hab + time)	26	3100.776	12.355247	0.0013546 9	1844.222
			Phi(~ecotype * rel_hab)p(~ecotype + rel_hab + time)	30	3103.994	15.573	0.0002710 9	1839.083
			Phi(~rel_hab + time)p(~ecotype * rel_hab + time)	46	3105.512	17.091474	0.0001268 8	1806.649
			Phi(~ecotype * rel_hab + time)p(~ecotype + rel_hab + time)	48	3105.523	17.101753	0.0001262 3	1802.355
ANG	D1	Full	Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab + time)	45	2227.473	0	0.3647654	1168.574
			Phi(~ecotype * rel_hab + time + thick)p(~ecotype * rel_hab + time)	46	2228.92	1.447726	0.1768658	2133.222
			Phi(~ecotype * rel_hab + time + weight)p(~ecotype * rel_hab + time)	46	2229.625	2.151926	0.1243737	2133.926
			Phi(~ecotype * rel_hab + time + PC1)p(~ecotype * rel_hab + time)	46	2229.633	2.160426	0.1238463	1168.575

			Phi(~ecotype * rel_hab + time + weight + thick)p(~ecotype * rel_hab + time)	47	2231.011	3.538052	0.06219211	2133.148
			Phi(~ecotype * rel_hab + time + thick + PC1)p(~ecotype * rel_hab + time)	47	2231.085	3.611852	0.05993905	2133.222
			Phi(~ecotype * rel_hab + time + weight + PC1)p(~ecotype * rel_hab + time)	47	2231.789	4.316052	0.04214973	2133.926
			Phi(~ecotype + rel_hab + time)p(~ecotype * rel_hab + time)	41	2232.936	5.463199	0.02375172	1182.643
			Phi(~ecotype * rel_hab + time + weight + thick + PC1)p(~ecotype * rel_hab + time)	48	2233.179	5.705887	0.02103759	2133.148
			Phi(~rel_hab + time)p(~ecotype * rel_hab + time)	39	2239.123	11.6502	0.00107697	1193.11
			Phi(~ecotype + time)p(~ecotype * rel_hab + time)	39	2252.707	25.2348	1.20878E-06	1206.695
			Phi(~ecotype * rel_hab)p(~ecotype * rel_hab + time)	30	2254.806	27.333659	4.23238E-07	1227.877
			Phi(~time)p(~ecotype * rel_hab + time)	37	2259.601	32.128335	3.84977E-08	1217.854
			Phi(~ecotype + rel_hab)p(~ecotype * rel_hab + time)	26	2261.164	33.691263	1.76217E-08	1242.623
			Phi(~rel_hab)p(~ecotype * rel_hab + time)	24	2267.943	40.470095	5.94353E-10	1253.575
			Phi(~ecotype)p(~ecotype * rel_hab + time)	24	2274.45	46.976895	2.29673E-11	1260.082
			Phi(~1)p(~ecotype * rel_hab + time)	22	2281.15	53.67762	8.05502E-13	1270.942
CZB	D1	Full	Phi(~ecotype * rel_hab + time + weight + thick)p(~ecotype * rel_hab + time)	54	3087.493	0	0.3478541	2974.801
			Phi(~ecotype + rel_hab)p(~ecotype * rel_hab + time)	30	3088.421	0.927617 3	0.2187598	1823.51
			Phi(~ecotype * rel_hab + time + weight + thick + PC1)p(~ecotype * rel_hab + time)	55	3089.693	2.199622 1	0.1158124	2974.823
			Phi(~ecotype * rel_hab + time + weight)p(~ecotype * rel_hab + time)	53	3089.965	2.472215 4	0.1010561	2979.448

			Phi(~ecotype * rel_hab + time)p(~ecotype * rel_hab + time)	52	3090.252	2.75836	0.08758441	1778.435
			Phi(~ecotype * rel_hab + time + thick)p(~ecotype * rel_hab + time)	53	3092.291	4.797615 4	0.03159426	2981.773
			Phi(~ecotype * rel_hab + time + weight + PC1)p(~ecotype * rel_hab + time)	54	3092.445	4.9521	0.02924571	2979.753
			Phi(~ecotype * rel_hab + time + PC1)p(~ecotype * rel_hab + time)	53	3092.456	4.962515 4	0.02909381	1778.469
			Phi(~rel_hab)p(~ecotype * rel_hab + time)	28	3094.295	6.801422 8	0.01160077	1833.568
			Phi(~ecotype * rel_hab + time + thick + PC1)p(~ecotype * rel_hab + time)	54	3094.524	7.0306	0.01034479	2981.832
			Phi(~ecotype)p(~ecotype * rel_hab + time)	28	3094.788	7.295222 8	0.00906274	1834.062
			Phi(~ecotype + rel_hab + time)p(~ecotype * rel_hab + time)	48	3095.38	7.886270 1	0.00674397	1792.212
			Phi(~ecotype + time)p(~ecotype * rel_hab + time)	46	3099.829	12.33529 14	0.00072916	1800.965
			Phi(~1)p(~ecotype * rel_hab + time)	26	3100.776	13.28286 46	0.000454	1844.222
			Phi(~rel_hab + time)p(~ecotype * rel_hab + time)	46	3105.512	18.01909 14	4.2521E-05	1806.649
			Phi(~ecotype * rel_hab)p(~ecotype * rel_hab + time)	34	3107.665	20.17185 67	1.4492E-05	1834.345
			Phi(~time)p(~ecotype * rel_hab + time)	44	3109.119	21.62560 51	7.0058E-06	1814.546
ANG	D2***	Crab ecotype	Phi(~rel_hab + time + thick)p(~rel_hab)	22	715.7335	0	0.2525974	669.0774
			Phi(~rel_hab + time)p(~rel_hab)	21	716.3352	0.60169	0.1869707	360.0566
			Phi(~rel_hab + time + weight + thick)p(~rel_hab)	23	716.4681	0.734555	0.1749533	667.5628

			Phi(~rel_hab)p(~rel_hab)	6	717.9072	2.173679	0.0851962	393.8358
			Phi(~rel_hab + time + thick + PC1)p(~rel_hab)	23	717.9894	2.255855	0.0817666	669.0841
			Phi(~rel_hab + time + weight)p(~rel_hab)	22	718.425	2.69148	0.065763	671.7689
			Phi(~rel_hab + time + PC1)p(~rel_hab)	22	718.5725	2.83901	0.0610866	360.0566
			Phi(~rel_hab + time + weight + thick + PC1)p(~rel_hab)	24	718.7291	2.995519	0.0564885	667.5628
			Phi(~rel_hab + time + weight + PC1)p(~rel_hab)	23	720.6741	4.940575	0.0213598	671.7689
			Phi(~rel_hab)p(~rel_hab + time)	18	722.3327	6.599165	0.0093205	372.6963
		Wave ecotype	Phi(~1)p(~rel_hab + time)	16	731.631	0	0.5564051	383.5387
			Phi(~rel_hab + time)p(~time)	31	734.8678	3.236876	0.110284	352.6334
			Phi(~rel_hab + time + thick)p(~time)	32	735.1012	3.470269	0.0981368	665.1012
			Phi(~rel_hab)p(~rel_hab + time)	18	735.8971	4.266112	0.0659199	383.4142
			Phi(~rel_hab + time + weight)p(~time)	32	736.7175	5.086549	0.0437382	666.7175
			Phi(~rel_hab + time + PC1)p(~time)	32	737.2202	5.589269	0.0340171	352.6062
			Phi(~rel_hab + time + weight + thick)p(~time)	33	737.447	5.816052	0.0303705	665.0539
			Phi(~rel_hab + time + thick + PC1)p(~time)	33	737.4944	5.863432	0.0296595	665.1012
			Phi(~rel_hab + time + weight + PC1)p(~time)	33	739.1107	7.479722	0.0132187	666.7175

			Phi(~rel_hab + time + weight + thick + PC1)p(~time)	34	739.8899	8.258989	0.0089531	665.09
		Hybrid ecotype	Phi(~1)p(~rel_hab + time)	16	822.7403	0	0.6886062	450.5276
			Phi(~rel_hab)p(~rel_hab + time)	18	824.8042	2.063861	0.245363	448.2418
			Phi(~rel_hab + time)p(~rel_hab + time)	33	830.2717	7.531444	0.0159418	419.676
			Phi(~time)p(~rel_hab)	19	830.785	8.044683	0.0123336	452.0318
			Phi(~rel_hab + time + weight)p(~rel_hab + time)	34	831.9058	9.165459	0.0070423	757.8343
			Phi(~rel_hab + time)p(~rel_hab)	21	831.9556	9.215302	0.006869	448.7883
			Phi(~rel_hab + time + thick)p(~rel_hab + time)	34	832.6173	9.877039	0.004934	758.5459
			Phi(~rel_hab + time + weight)p(~rel_hab)	22	833.5743	10.83401	0.0030577	787.0694
			Phi(~rel_hab + time + thick)p(~rel_hab)	22	834.0876	11.34726	0.0023656	787.5826
			Phi(~rel_hab + time + PC1)p(~rel_hab)	22	834.177	11.43667	0.0022622	448.7862
		Crab release habitat	Phi(~ecotype + time)p(~ecotype)	21	659.0131	0	0.4189	388.5101
			Phi(~ecotype + time + thick)p(~ecotype)	22	661.2249	2.211783	0.1386206	612.8057
			Phi(~ecotype + time + weight)p(~ecotype)	22	662.0998	3.086613	0.0895078	613.6805
			Phi(~ecotype + time + PC1)p(~ecotype)	22	662.1064	3.093243	0.0892116	389.2015

			Phi(~ecotype + time + thick + PC1)p(~ecotype)	23	663.6478	4.634684	0.0412764	612.8057
			Phi(~ecotype + time + weight + thick)p(~ecotype)	23	664.514	5.500884	0.0267676	613.6719
			Phi(~ecotype + time + weight + PC1)p(~ecotype)	23	664.6955	5.682384	0.0244454	613.8534
			Phi(~ecotype + time + weight)p(~1)	20	665.0074	5.994262	0.0209157	621.371
			Phi(~ecotype + time)p(~1)	19	665.0085	5.995401	0.0209038	399.247
			Phi(~ecotype + time + PC1)p(~1)	20	665.0169	6.003762	0.0208166	396.8949
		Wave release habitat	Phi(~ecotype)p(~ecotype + time)	18	501.7781	0	0.2563226	256.6402
			Phi(~ecotype)p(~time)	16	502.2994	0.521337	0.1975059	261.6811
			Phi(~1)p(~time)	14	502.8845	1.106388	0.1474137	266.7213
			Phi(~1)p(~ecotype + time)	16	503.0166	1.238527	0.137989	262.3983
			Phi(~ecotype + time + thick)p(~ecotype)	22	504.9388	3.160716	0.0527772	457.2318
			Phi(~ecotype + time)p(~ecotype)	21	505.1844	3.406279	0.0466792	253.1436
			Phi(~time)p(~ecotype)	19	505.3777	3.599649	0.0423773	257.9556
			Phi(~ecotype + time + thick + PC1)p(~ecotype)	23	507.2907	5.512589	0.0162833	457.2319
			Phi(~ecotype + time + PC1)p(~ecotype)	22	507.5191	5.740976	0.0145261	253.1436

			Phi(~ecotype + time + thick)p(~ecotype + time)	34	508.0089	6.23081	0.0113706	430.8901
		Hybrid release habitat	Phi(~ecotype + time)p(~ecotype + time)	33	1091.892	0	0.3650062	488.1474
			Phi(~ecotype + time + thick)p(~ecotype + time)	34	1093.522	1.629841	0.1615789	1021.762
			Phi(~ecotype + time + PC1)p(~ecotype + time)	34	1094.113	2.220441	0.1202645	488.1474
			Phi(~ecotype + time + weight)p(~ecotype + time)	34	1094.121	2.228841	0.1197605	1022.361
			Phi(~ecotype + time + weight + thick)p(~ecotype + time)	35	1095.101	3.20851	0.0733806	1021.114
			Phi(~ecotype + time + thick + PC1)p(~ecotype + time)	35	1095.75	3.85731	0.0530513	1021.762
			Phi(~ecotype + time + weight + PC1)p(~ecotype + time)	35	1096.191	4.29871	0.0425449	1022.204
			Phi(~ecotype)p(~ecotype + time)	18	1097.192	5.299497	0.0257946	525.9325
			Phi(~ecotype + time + weight + thick + PC1)p(~ecotype + time)	36	1097.335	5.443038	0.0240082	1021.114
			Phi(~ecotype + time)p(~time)	31	1101.376	9.483465	0.0031841	502.0508
CZB	D2***	Crab ecotype	Phi(~rel_hab)p(~rel_hab + time)	22	1002.402	0	0.619204	563.3252
			Phi(~1)p(~rel_hab + time)	20	1003.971	1.568838	0.282595	569.313
			Phi(~rel_hab + time)p(~rel_hab + time)	40	1008.604	6.20136	0.027876	527.7768
			Phi(~rel_hab + time + weight)p(~rel_hab + time)	41	1008.664	6.261175	0.027054	918.0751

			Phi(~rel_hab + time + weight + thick)p(~rel_hab + time)	42	1010.383	7.980476	0.011452	917.3529
			Phi(~rel_hab + time + thick)p(~rel_hab + time)	41	1010.626	8.223985	0.01014	920.0379
			Phi(~rel_hab + time + PC1)p(~rel_hab + time)	41	1011.032	8.629185	0.00828	527.7753
			Phi(~rel_hab + time + weight + PC1)p(~rel_hab + time)	42	1011.431	9.028886	0.00678	918.4013
			Phi(~rel_hab + time + weight + thick + PC1)p(~rel_hab + time)	43	1012.837	10.4342	0.003358	917.3529
			Phi(~rel_hab + time + thick + PC1)p(~rel_hab + time)	42	1013.069	10.66655	0.00299	920.039
		Wave ecotype	Phi(~1)p(~rel_hab + time)	20	989.9445	0	0.71326	567.206
			Phi(~rel_hab)p(~rel_hab + time)	22	991.767	1.822565	0.286736	564.5847
			Phi(~time)p(~rel_hab + time)	38	1015.35	25.40556	2.17E-06	550.9373
			Phi(~rel_hab + time + thick)p(~rel_hab + time)	41	1018.994	29.0491	3.51E-07	927.8825
			Phi(~rel_hab + time + PC1)p(~rel_hab + time)	41	1019.356	29.41154	2.93E-07	547.6117
			Phi(~rel_hab)p(~time)	20	1020.919	30.97417	1.34E-07	598.1802
			Phi(~rel_hab + time + weight + thick)p(~rel_hab + time)	42	1020.919	30.97473	1.34E-07	927.3383
			Phi(~rel_hab + time + weight + PC1)p(~rel_hab + time)	42	1020.927	30.98206	1.34E-07	927.3456
			Phi(~rel_hab + time + thick + PC1)p(~rel_hab + time)	42	1021.463	31.51889	1.02E-07	927.8825
			Phi(~rel_hab + time)p(~rel_hab + time)	40	1021.739	31.79455	8.9E-08	552.4515

		Hybrid ecotype	Phi(~rel_hab)p(~time)	20	1120.495	0	0.795438	648.4046
			Phi(~1)p(~time)	18	1123.629	3.13399	0.165985	655.9028
			Phi(~1)p(~rel_hab + time)	20	1127.519	7.0243	0.02373	655.429
			Phi(~rel_hab)p(~rel_hab + time)	22	1128.46	7.96504	0.014826	651.9654
			Phi(~rel_hab + time)p(~time)	38	1144.227	23.73209	5.59E-06	630.985
			Phi(~rel_hab + time + thick)p(~time)	39	1144.689	24.19422	4.44E-06	1059.225
			Phi(~rel_hab + time + weight + thick)p(~time)	40	1146.438	25.94301	1.85E-06	1058.573
			Phi(~rel_hab + time + weight)p(~time)	39	1146.47	25.97502	1.82E-06	1061.006
			Phi(~rel_hab + time + PC1)p(~time)	39	1146.616	26.12122	1.69E-06	630.984
			Phi(~rel_hab + time + thick + PC1)p(~time)	40	1147.091	26.59601	1.33E-06	1059.226
		Crab release habitat	Phi(~ecotype)p(~ecotype + time)	22	746.8674	0	0.977483	447.623
			Phi(~ecotype)p(~time)	20	754.9255	8.058152	0.01739	460.4171
			Phi(~1)p(~ecotype + time)	20	757.3829	10.51549	0.00509	462.8745
			Phi(~ecotype + time + weight)p(~ecotype + time)	41	770.0748	23.20746	8.93E-06	672.6998
			Phi(~ecotype + time)p(~ecotype + time)	40	770.2733	23.40596	8.08E-06	424.6157

			Phi(~ecotype + time + thick)p(~ecotype + time)	41	771.9814	25.11399	3.44E-06	674.6064
			Phi(~1)p(~time)	18	772.6225	25.7551	2.5E-06	482.7734
			Phi(~ecotype + time + weight + thick)p(~ecotype + time)	42	772.8967	26.02939	2.18E-06	672.6994
			Phi(~ecotype + time + weight + PC1)p(~ecotype + time)	42	772.8971	26.02977	2.18E-06	672.6998
			Phi(~ecotype + time + PC1)p(~ecotype + time)	41	773.0705	26.20314	2E-06	424.6157
		Wave release habitat	Phi(~1)p(~time)	18	716.9369	0	0.40764	421.3671
			Phi(~ecotype)p(~time)	20	719.0433	2.106461	0.142189	418.9928
			Phi(~ecotype + time)p(~ecotype + time)	40	719.6031	2.666272	0.107474	371.5959
			Phi(~1)p(~ecotype + time)	20	720.0314	3.094491	0.086759	419.9808
			Phi(~ecotype + time)p(~time)	38	720.5314	3.594513	0.067568	377.5938
			Phi(~ecotype)p(~ecotype + time)	22	721.5348	4.597891	0.040913	416.9493
			Phi(~ecotype + time + thick)p(~ecotype + time)	41	722.5002	5.56334	0.025247	629.4617
			Phi(~ecotype + time + weight)p(~time)	39	722.7951	5.858225	0.021786	634.8588
			Phi(~ecotype + time + thick)p(~time)	39	723.047	6.110135	0.019208	635.1107
			Phi(~ecotype + time + PC1)p(~ecotype + time)	41	723.1825	6.24562	0.01795	372.616

		Hybrid release habitat	Phi(~ecotype)p(~ecotype + time)	22	1563.562	0	0.701407	823.2079
			Phi(~1)p(~ecotype + time)	20	1565.441	1.879169	0.274103	829.3445
			Phi(~ecotype)p(~time)	20	1570.471	6.908869	0.022168	834.3741
			Phi(~1)p(~time)	18	1576.051	12.48891	0.001362	844.1865
			Phi(~ecotype + time)p(~ecotype + time)	40	1579.234	15.67237	0.000277	799.4033
			Phi(~ecotype + time + weight)p(~ecotype + time)	41	1580.169	16.60658	0.000174	1492.943
			Phi(~ecotype + time + weight + thick)p(~ecotype + time)	42	1580.804	17.24234	0.000126	1491.315
			Phi(~ecotype + time + thick)p(~ecotype + time)	41	1581.433	17.87138	9.23E-05	1494.207
			Phi(~ecotype + time + PC1)p(~ecotype + time)	41	1581.482	17.92008	9.01E-05	799.3945
			Phi(~ecotype + time + weight + PC1)p(~ecotype + time)	42	1582.425	18.86274	5.62E-05	1492.935
Abbreviations: Rel_hab: release habitat; thick: thickness; PC1: waves								