Why aggregate?

Thesis submitted by

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Abstract

By promoting coexistence, aggregation has been identified as a major source of biodiversity in insects. This study into the evolution of aggregation in *Drosophila* produced novel insights into the mechanisms and explanations underlying aggregation behaviour.

1. The conclusions of a literature review together with experimental results suggest that mammals are unlikely to affect insect densities in resources while birds have the potential to do so.

2. Allee effects occur in *D. simulans* but they are highly dependent on the precise properties of the resource. Mould proved unlikely to mediate Allee effects The relationship between yeast and larvae is more complex than hitherto assumed; competitive interactions may be responsible for the occurrence of Allee effects.

3. Oviposition in *D. simulans* is non-random and dependent on environmental properties (light) and characteristics of the resource (accessibility or detectabitliy, size of oviposition surface). Females do not respond to the size of resource units.

4. Individual oviposition patterns are highly variable and difficult to select for. Egg distributions generated by isolated flies are the products of different clutches.

5. Male presence and pre-experimental adult density have little effect on *D. simulans* oviposition behaviour. Females lay more eggs that are more aggregated on high quality substrates compared to those of lower quality. Females avoid using sites already containing eggs on natural substrates but still generate aggregated egg distributions. Resource use overlap can be increased by reducing the number of high quality patches. Egg distributions of *D. simulans* and *D. melanogaster* are randomly associated.

6. Females lay fewer, more scattered eggs on grapes with high compared to low sugar concentrations but only if yeast is present. Higher sugar content increases survivorship and adult body size. Female oviposition site choice reflects the quality of the substrate in terms of offspring survival and size. Combined with density-dependent effects this indicates that oviposition choice is a problem of optimal foraging strategy.

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Declaration

I declare that the work contained in this thesis is the result of my own work and was written entirely by myself.

Nothing in biology makes sense except in the light of evolution.

Theodosius H. Dobzhansky
1973

General introduction

Ecologists have long been interested in explaining the species richness, or biodiversity, in ecological communities. There are various ways in which one can approach the question of how communities are structured; some emphasise abiotic or environmental factors, while others stress the importance of biological interactions (classified e.g. by Cornell & Lawton 1992; see also Shorrocks & Sevenster 1995). In interactive communities, strong biotic interactions occur between species at the same trophic level and interactive communities have traditionally held a central place in community theory (see Cornell & Lawton 1992). Although there is some debate over how important biotic interactions are in structuring communities or, indeed, on which spatial scale one should consider the problem (e.g. Caswell 1976; Connor & Simberloff 1979; Lawton 1982; Strong *et al.* 1984; Cornell & Lawton 1992), traditionally the view has been held that species interactions, especially competition, are important factors in influencing the species richness of communities (e.g. MacArthur 1972; Cody 1975). In a review of over 150 published field studies, Schoener (1983) concluded that competition was found in 76% of species examined. If species were to persist within a community, it was assumed that their ecological requirements, or niches, had to become partitioned. Species whose niches showed too much overlap would exclude each other competitively, depending on their relative competitive abilities (see Lotka 1925; Volterra 1928; Hardin 1960). Later this was generalised to the statement that n species could not coexist on fewer than n resources (e.g. MacArthur & Levins 1964). The mechanisms by which niche space in communities can be partitioned have been reviewed, for example, by Wiens (1989). Frequently though, communities show a large degree of diversity even within groups of very similar species that show little niche differentiation and that are able to coexist (e.g. Hutchinson 1961; Strong 1982; Strong *et al.* 1984). Further, in some communities the number of coexisting species was found to exceed the number of limiting resources; this was termed by Hutchinson (1961) the 'paradox of diversity'. Numerous mechanisms have been proposed that can help explain the large number of coexisting species, without necessarily assuming a large degree of resource heterogeneity and niche partitioning; these include, for example, temporal processes, predator-mediated coexistence, environmental disturbance and spatial processes (see Shorrocks 1990; Hanski 1990; Chesson 1991 or Cornell &

Lawton 1992 for recent reviews). More recently, the potential of space as an important explanatory concept in community theory has become recognised and more research is now focused on spatial dynamics (see Tilman & Kareiva 1997). The role of spatial avoidance as a mechanism that promotes coexistence presents the background to this work.

Coexistence through spatial avoidance works only if competing species are distributed in such a way that the intensity of interspecific relative to intraspecific interactions is reduced (e.g. Ives & May 1985). One of the very early approaches to such a mechanism was published by Shorrocks *et al.* (1979; see also Atkinson & Shorrocks 1981; *et seq.*). They demonstrated through a simulation model that an inferior competitor could persist if the competing life-stages of a superior competitor have an aggregated utilisation of fragmented environments (see also Shorrocks & Rosewell 1986; Rosewell *et al.* 1990); the system simulated was insects breeding in ephemeral patches of food. There was anecdotal evidence that discrete and ephemeral breeding sites supported very diverse insect communities (e.g. Elton 1966; Beaver 1977; later reviewed by Atkinson & Shorrocks 1984; Atkinson 1985). In most insects, competition is largely confined to the larval stage and the aggregation of eggs and hence larvae is very widely observed (e.g. Grimaldi & Jaenike 1984; Atkinson & Shorrocks 1984; Hanski 1987; Ives 1988; Rosewell *et al.* 1990). Rosewell *et al.* (1990), for example, showed that in a data set consisting of 360 dipteran species that utilise patchy and ephemeral resources, 90% showed a significant degree of aggregation. Breeding in such resources is a very general life-style: typical resources include fruit, fungi, carrion and dung (reviewed in Shorrocks & Rosewell 1987; Rosewell *et al.* 1990). Shorrocks *et al.* (1979; and see Atkinson & Shorrocks 1981; *et seq.*) argued that aggregation over such a single, patchy and ephemeral resource type may permit coexistence of competing species, known today as the 'aggregation model of coexistence'. This simulation mimics the competitive interactions of a two-species *Drosophila* system where eggs are aggregated over patches (mushrooms or fruit) according to a negative binomial distribution. An important prediction of this model is that as long as competing species aggregate their larvae independently over the patches, an inferior competitor can persist in the 'probability refuges' created by the aggregated distribution of the superior competitor. For realistic degrees of aggregation measured both in the laboratory and in the field, the model also predicts that coexistence should be the rule rather than the exception. These conclusions are supported by evidence from laboratory studies (Shorrocks 1991) and

field sampling or manipulations (Atkinson & Shorrocks 1984; Atkinson 1985; Ives 1988; 1991; Kouki & Hanski 1995).

Some aspects of the original model have been criticised (e.g. Green 1986) and the model has been refined by several different approaches (e.g. Ives & May 1985; Ives 1988; 1991). Other models that differ in some of the underlying assumptions, especially the way the aggregated distributions are generated in the simulation, have been developed (e.g. Hanski 1981; De Jong 1982) and extended (Sevenster 1996 who also presents an excellent review of the background theory), and the spatial scale at which aggregation promotes coexistence has also been considered (Inouye 1999). The two species system of the original model has been extended to generate a 'guild model' that could predict the number of species that can coexist without resource partitioning, thereby placing the emphasis on the wider insect community (Shorrocks & Rosewell 1986; 1987).

Despite the differences in the approach, the general consensus is that the original conclusions of the aggregation model are robust; aggregation can facilitate coexistence and the degree of aggregation found in insect systems appears to be able to explain coexistence of competitors (see Sevenster 1996). While this is widely accepted, the mechanisms by which aggregation is generated remain the subject of some debate (Green 1986; Sevenster 1996). More importantly perhaps, surprisingly little is understood that could explain aggregation in an evolutionary context. A pertinent question to ask might be, 'why should organisms aggregate at all?'. Expectations from competition theory predict that competition between individuals must be more intense, the more their ecological requirements match. It is not then intuitive why a strategy which enhances the intensity of competition between conspecifics should evolve over one that reduces it. The point was reinforced by recent theoretical work (Dytham & Shorrocks 1992; 1995) which indicated that aggregation may be a disadvantageous strategy that is susceptible to invasion and exclusion by a non-aggregating strategy. Clearly, there is a discrepancy: aggregation is extremely prevalent in nature, yet it appears disadvantageous in models that are based on parameters reflecting our understanding of the system to date.

This issue has received very little attention. Ives (1988; 1991) reported, in an aside to his study, that aggregation is probably caused by qualitative differences between patches

to which ovipositing females respond. Sevenster (1996) sums up the current understanding in stating that "in nature, variation in quality and conspicuousness between patches should be the rule rather than the exception" and that "this will explain much of the aggregation of ovipositing females". There is, however, little evidence that this is the sole or, indeed, the most important explanation for aggregation. Before focusing on its adaptive significance, it is imperative to consider the heritability, or genetic basis of aggregation. Such a basis has been demonstrated by del Solar (1968) who showed that selection could significantly alter the degree to which female *Drosophila pseudoobscura* aggregated their eggs; later Ruiz & del Solar (1986) also confirmed this for *D. melanogaster* in a divergent, mass selection experiment that produced strains, with high and low tendencies to aggregate. The chromosomal analysis of the genetic system controlling aggregation has shown it to be polygenic with a high degree of additive variance (Ruiz & del Solar 1993). The genes for aggregation are distributed over chromosomes II and III in the *Drosophila* genome (Ruiz-Dubreuil & Köhler 1994). Ruiz-Dubreuil & Köhler (1994) argue that the analysis of the genetic system, revealing a dominance component directed towards an increase in aggregation, suggest that the degree of aggregation is of ecological importance.

To address the question of the ecological and evolutionary importance of aggregation was the aim of this study. The discrepancy between the expected predictions of simulations and the observed occurrence of the phenomenon in nature indicates that our understanding of the system is still inadequate. The adaptive significance of aggregation is ill understood and it is unclear whether there are selective forces that favour aggregation in some way, what selective processes could be involved, or, indeed, whether there is some fundamental aspect to insect oviposition behaviour that may make aggregated egg distributions inevitable. My work represents a laboratory-based investigation of aggregation processes in *Drosophila*. The study has two, main focal points. Interactions with predators as putative selective forces towards aggregation are considered first but the main investigation emphasises the intra-specific interactions, using mainly the fruit-breeding *Drosophila simulans* Sturtevant. Although the process of aggregation, as it is understood, is a phenomenon of a group of conspecifics, in seeking evolutionary explanations we must examine the individual. Shorrocks & Bingley (1990) quoted from a paper entitled 'From individual behaviour to population dynamics' by Hassel & May (1985) which is worth re-quoting in the context of this project: "...those situations where a phenomenological description of the way

subpopulations interact in a spatially heterogeneous environment can, on the one hand, be grounded on an understanding of the behaviour of individuals and can, on the other hand, lead to insights about population dynamics and community structure". Yet, an understanding of the individual (oviposition) behaviour of aggregating insects is "sadly lacking" (Shorrocks & Bingley 1990) and this is the main focus of the work presented here.

Chapter 1 - Preliminary study: Age- and size related oviposition patterns in *D. simulans*

Summary

Daily egg output of 60 *D. simulans* females was investigated on plates of grape pulp for the first ten days after eclosion. A small number of females started oviposition within the first 24 hours after eclosion; daily egg output for the population increased rapidly over the first four days of life, levelled off by day 5 and remained constant until day 10. The population mean from day 5 onwards was 48.74 eggs per female in 24 hours (SD = 19.25). There was a high degree of variation in oviposition rates, most of which was size-dependent: larger females laid eggs earlier, produced more eggs younger and in total over the first 10 days from eclosion.

Introduction

Before starting the investigation into the evolution of aggregation in *Drosophila*, a pilot study was conducted. This had two aims: 1) To establish the patterns of reproductive output in *D. simulans*, on an optimal substrate, early during the adult life-span. This would allow comparisons during the course of the study whenever changes in oviposition behaviour were considered and justify decisions over ageing-regimes during the investigation. 2) To gain a measure of size-related progeny production early in the adult life-span. This would permit more conclusive statements when assessing differences in fitness characters other than survivorship, e.g. size-related fecundity of offspring, necessary to any study of evolutionary strategies in *Drosophila*.

Breeding success in *Drosophila* is influenced by many variables, such as age, reproductive effort and body size (reviewed by Partridge 1988). After adults eclose from the puparium, there is a period without any reproductive activity, the duration of which is variable between species and also between the sexes (e.g. Donegan 1984). In *Drosophila melanogaster* this typically lasts 12 hours for males and 12-14 hours for females (Ashburner 1989) and is likely to be very similar in the closely related *Drosophila simulans*. The relationship between age, size and reproductive effort in *Drosophila* is well documented (e.g. Tantawy & Vetukhiv 1960; Partridge *et al.* 1988). David *et al.*(1974) showed that daily egg production in *D. melanogaster* females peaked

at 10 days and then declined fairly rapidly. Although the mortality rate increases rapidly with ageing (more than 70% are dead by 37-40 days, Partridge *et al.*1986), surviving females in the study of David *et al.*(1974) continued to lay eggs up to about 50 days. While all these studies were conducted in the laboratory, it is very important to consider survival rates in natural populations, especially when the aim is to draw valid conclusions about evolutionary strategies. Rosewell & Shorrocks (1987) reported that, based on capture-recapture data, the seven species of *Drosophila* examined in their study, including *Drosophila simulans* and *D. melanogaster,* could expect to live between 1.3 and 6.2 (mean = 2.8) days in the field. Thus, while flies can have a high total reproductive output over the whole duration of their laboratory lives, in the field early offspring production would appear to be the most important.

Body size is a major factor in reproductive success in *Drosophila*, mediated through the effect of size on mating success, longevity and fertility with relationships that are generally positively correlated (see Partridge 1988). Size differences can be attributed to a variety of factors other than simply inheritance (e.g. Robertson 1957) and include: temperature (Thomas 1993; Anderson 1973), nutrition (Thomas 1993), larval crowding and the nature of the breeding site (Sang 1949; Atkinson 1979). In females, lifetime progeny production increases significantly and linearly with increasing size (e.g. Robertson 1957; Tantawy & Rakha 1964). In a project concerned with evolutionary strategies it was thought necessary to establish differences in size-related progeny production early in the *D. simulans* adult life-span, especially considering the short life-expectancy in the field.

Materials & Methods

The egg collecting apparatus consisted of a clear plastic chamber (approx. 500ml) with ventilation through a cotton wool bung at the top and with sand (100g) at the base. Sand was moistened daily (30ml water) to keep humidity favourable. The egg collecting medium was made up of 1% agar-water solution topped with a set amount of grape pulp (2.5 ml). The pulp was obtained by liquidizing grapes (Spanish seedless variety) which was then boiled to kill most microbial contamination and frozen. To control for factors which could affect oviposition behaviour or rates, the grape pulp used throughout the experiment originated from the same bunch of grapes. Prior to each trial, food plugs were prepared by pouring 10 ml of 1% agar solution into small, circular

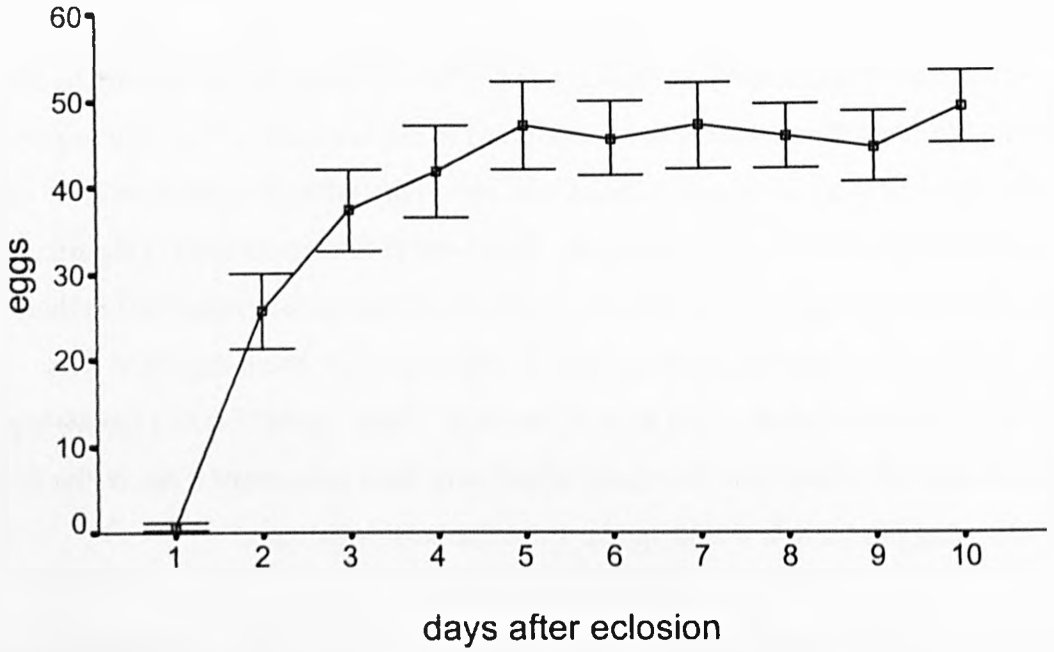

Fig. 1.1 Mean (95% C.I. based on s.e. mean) daily eggs output of 60 female *D. simulans* over the first 10 days of adult life.

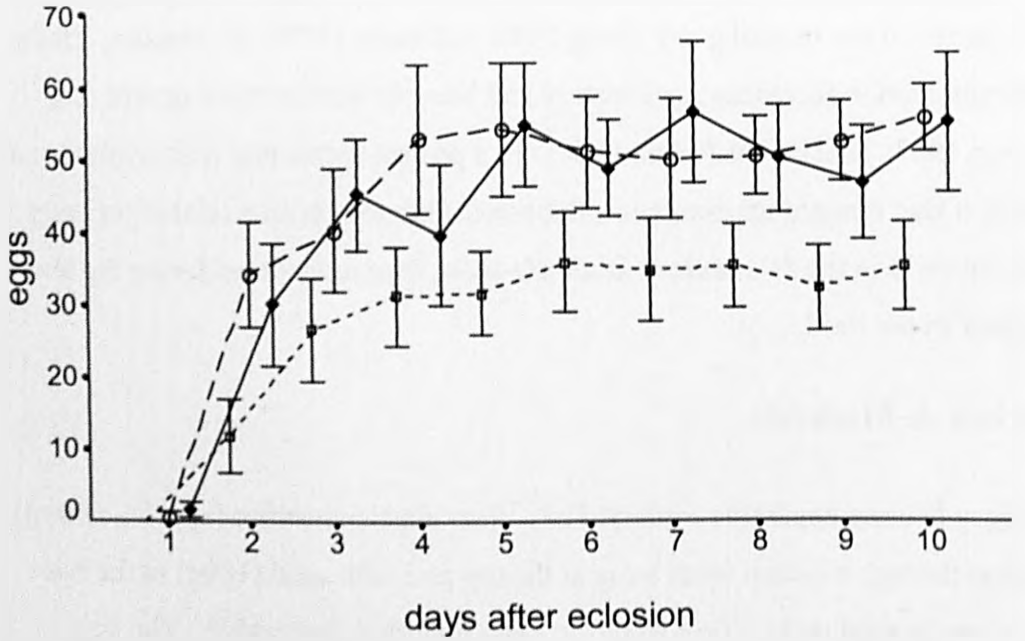

Fig. 1.2. Mean (± 95% C.I. based on s.e. mean) daily egg output of female *D. simulans* in different size classes over the first 10 days after eclosion. open square = class 1 (N = 20), open circle = class 2 (N = 20), solid diamond = class 3 (N = 20).

plastic receptacles (20 mm diameter, 1 cm deep) that was then left to set. Grape pulp was defrosted and 2 drops (approx. 1 ml) were placed onto the agar base, spread and allowed to dry off for 2 hours. One food plug was placed at the bottom of a plastic chamber to complete the egg collecting apparatus. For each trial, newly (less than 5 hours old) eclosed female *D. simulans* were anaesthetised, using CO_2, and transferred individually into a chamber together with two sexually mature males. All flies had been reared on standard *Drosophila* medium (Ashburner & Thompson 1978) in 25 x 75 mm glass vials. Chambers were placed into a cooled incubator at 25 ± 0.5°C with a 12 /12 hour light/dark cycle and left undisturbed. Every 24 hours, food plugs were removed and replaced with an identical fresh plug without using further anaesthetics. Using a binocular microscope, the exact number of eggs per plug was counted and recorded. The procedure was repeated over 10 days. Any males dying during the 10 day trial were replaced; death or escape of the female terminated the trial. At the end of a trial, females were killed by placing them into 70 %ethanol for storage. Later, they were laid on their side and their thorax lengths were measured to the nearest 0.01 mm, from the base of the most anterior humeral bristle on the margin of the mesothorax to the distal midpoint of the scutellum. Thorax length is known to be a good measure of body size since it shows positive phenotypic and genetic correlations with other size measures such as tibia and wing lengths or body weight (e.g. Robertson 1963; Wilkinson *et al.* 1990). 64 females were examined in total; 4 died during a trial so that the final data set used in subsequent analyses consisted of 60 flies.

Results

Over the ten days, females laid 379.48 (SD = 160.97) eggs on average, the smallest number of eggs laid was 42 while the maximum was 782. Mean egg output increased rapidly over the first three days before reaching a maximum on day 5 (Fig. 1.1). The average thorax length of all females was 0.98 mm (SD = 0.07). To analyse size-dependent egg output females were divided into three size classes based on percentiles: class 1 = size range 0.80 mm - 0.97 mm, class 2 = 0.98 mm - 1.01 mm and class 3 = 1.02 mm - 1.12 mm. Females in class 3 laid eggs earlier than those of the other two classes (one-way ANOVA for eggs on day 1; SS = 30.34, d.f. = 2, F = 4.88, p = 0.011). On subsequent days, egg output was consistently and mostly highly significantly larger in classes 2 and 3 (Fig. 1.2). This result is reflected in the highly significant regression

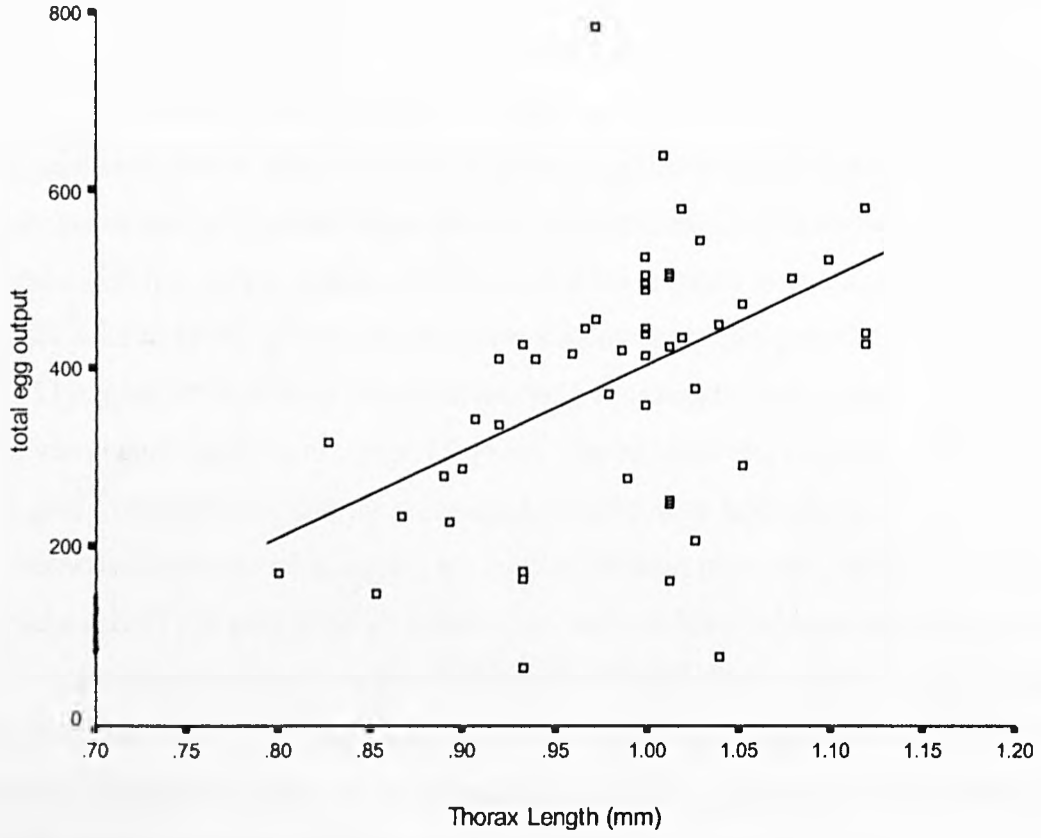

Fig. 1.3 Total number of eggs laid by *D. simulans* females over the first 10 days after eclosion, plotted against thorax length ($r^2 = 0.228$).

($r^2 = 0.228$, $p < 0.001$) for thorax length and total egg numbers (all 10 days; Fig. 1.3). Oviposition rates varied from day to day. The mean variation calculated as the coefficient of variation (V) (this measure expresses the standard deviation as a percentage of the mean thus making it possible to compare variation amongst samples of very different means; Sokal & Rohlf 1995) for the entire population was quite high at 55.63% and varied from a minimum of 35.38% to 141.28% maximum. However, when the first three days during which oviposition rates clearly changed were excluded, V = 29.74%. Females still differed substantially (range of $V = 96.48$) but the degree of variation was not size-dependent (one-way ANOVA on V for size classes; SS = 769.8495, d.f. = 2, F = 1.4358 ns).

Discussion

The observation that daily egg output reached its maximum after 4-5 days with little change over subsequent days is very similar to the results obtained by Boulétreau-Merle (1971) for *D. melanogaster* and shows that the two sibling species have very similar profiles. It is known that *D. melanogaster* and *D. simulans* females are limited in the numbers of eggs that can be laid in a certain time period by the actual physiological processes involved in oogenesis, or more precisely, in choriogenesis (Ashburner 1989). It has been shown in *D. melanogaster* that females are limited to laying around 100 eggs in 24 hours (see Ashburner 1989). Although female *D. simulans* in these experiments laid egg numbers generally below 100, as many as 130 eggs were sometimes laid. This indicates that in *D. simulans* a slightly faster oviposition rate is possible and that the medium (grape pulp with yeast) was highly suitable.

The results confirm that body size is intimately related to fecundity in female *Drosophila* (Robertson 1957; Tantawy & Vetukhiv 1960). Tantawy & Vetukhiv (1960) concluded that the often observed increase in egg production with increasing body size (or weight) may indicate that the two characters are genetically correlated. There is ample evidence that size-related oviposition is part of general life-history strategy differences. Tantawy (1961) showed that larger female *D. pseudoobscura* lived longer and laid more eggs than smaller females. Partridge & Fowler (1992) however, found that there were significant differences in lifetime egg production for different lines selected for longevity and for late fecundity ; long-lived lines were heavier and

produced more eggs but were older at eclosion. Hillesheim & Stearns (1992) demonstrated that in *D. melanogaster*, larger females laid more eggs early in life and lived for a shorter time than smaller flies, indicating that there is a cost associated with this early increased reproduction. My results for *D. simulans* confirmed the early increase in egg production for larger flies compared to smaller ones. A lot more of the genetic details have been worked out since the earlier studies (e.g. Buck *et al.* 1993) and the issue of larger size, early fecundity but shortened longevity still receives much attention, mainly in research concerned with ageing and trade-off scenarios (e.g. Zwan *et al.* 1995; Stearns & Kaiser 1996; Nunney 1996). In *Drosophila*, it must remain questionable how important a long term strategy really is; since the life-span in the field is assumed to be short (Rosewell & Shorrocks 1987). It would appear that early reproductive effort is more crucial than that later in life.

Besides fitting into the argument over differences in life-history strategies, the experiment clearly fulfilled its purpose in the context of the proposed study. Oviposition rates in *D. simulans* increase rapidly over the first five days of life but then level off and remain very steady until at least day 10. Additionally, females readily oviposit on grape pulp, even if reared on standard culture medium, with an average daily egg output of around 50. In subsequent studies it would be possible to compare oviposition patterns to these standards.

Chapter 2 - Vertebrate predation - a factor in aggregation?

Summary

Insectivorous vertebrates have a clear impact on their prey. Many nominally frugivorous or granivorous vertebrates have a less obvious impact on insects yet their feeding preferences are likely to affect the behaviour and ecology of insects that utilise shared resources. Research on such interactions has rarely considered these effects. Here, I review the extent of interaction between insects and non-insectivorous vertebrates identifying both competition for resources and incidental predation by vertebrates. The effect of insect infestation on vertebrates shows some general patterns: most vertebrates discriminate between infested and non-infested resources, and while mammals either ignore insects or actively choose infested fruits, birds generally avoid infestation. Further to mechanisms conventionally proposed, some insects may aggregate to avoid predation.

To test whether vertebrates would not only respond to the presence or absence of insects but also to their density, small mammals and common British garden birds were presented with apple infested with a range of densities of *Drosophila* larvae. Field mice and bank voles showed no discrimination. Some birds clearly avoided infested fruits and responded to infestation densities.

Introduction

The resources utilised as breeding and feeding sites by many insects are also favoured as food sources by larger, vertebrate species. Many species of Diptera and Coleoptera use fruits, fungal fruiting bodies, seeds or carrion as sites for oviposition and larval development (e.g. Elton 1966; Shorrocks and Rosewell 1987). As many bird and mammal species include such resources in their diets, they can act both as competitors by reducing the number of available breeding sites (e.g. Sikes 1996) and as predators by killing eggs and larvae (e.g. Drew 1987). While such predation may be incidental because insects are simply ingested along with the resource, it could also be the result of an active choice. Insects are a valuable food source; they have a high fat content and are

rich in proteins and amino acids and can make up for deficiencies in, for example, fruit pulp (Redford and Dorea 1984). Many insects have characters that probably evolved as strategies to avoid vertebrate-associated mortality and competition. Some, for example, are immune to digestion (Chung and Waller 1986, Guix and Ruiz 1995) while others make resources less palatable or attractive by altering physical and chemical properties, such as colour or taste (Carter 1939, Manzur and Courtney 1984, Krischik *et al.* 1989), by deposition of excreta (see Traveset *et al.* 1995), by introduction of micro-organisms associated with rot (Janzen 1969) or by arresting fruit ripening (Krischik *et al.* 1989, Kreuger and Potter 1994). Leaving the resource early to pupate in the soil also avoids ingestion (e.g. Drew 1987). Whether these strategies have evolved as adaptations to vertebrate feeding behaviour is likely to depend on the extent of resource use overlap and the evolutionary history of the interactions (Sallabanks and Courtney 1992). Strikingly, many, though by no means all, insects that share resources with vertebrates tend to have very clumped distributions, i.e. they aggregate their eggs and larvae over the resources which are generally patchy and ephemeral in the environment. This, for example, is very commonly observed in Diptera breeding in fruit, fungi (Shorrocks 1990) and carrion (Ives 1988, 1991). Vertebrate frugivores, scavengers, those feeding on fungi and possibly granivores are thus often presented with resources that contain not only one or few but often high densities of insect eggs and larvae.

Interactions between vertebrates and invertebrates have been investigated mainly on fruits and seeds due to their importance both in ecological (plant-disperser interactions) and economical terms (insects as fruit pests). The many ways in which the ecology and evolution of fruit-feeding insects are linked closely to plant-vertebrate associations have been reviewed by Sallabanks and Courtney (1992) but the impact on the insects themselves rather than on the plant-disperser system has been largely overlooked.

This study aims to consider this impact more closely, particularly on insects that aggregate. The extent of resource use overlap between vertebrates and invertebrates in published studies is assessed and I identified how often vertebrates are deterred or attracted to insect-infested resources or do not discriminate between them. Finally, a study is presented that investigates whether vertebrates are likely to respond not only to presence or absence of insect larvae but also to their density in infested resources, leading on to a consideration of whether aggregation in itself could function as a strategy by which insects avoid predation.

Resource use overlap

In order for vertebrate frugivory to exert any selection pressure on insects, the frequency of interactions on the shared resource or resources has to be sufficiently high. Thorough examination of the literature revealed little evidence for this with the resource types and insect groups commonly associated with aggregation behaviour. However, many studies involve unidentified insects that may aggregate or resources suitable for aggregating insects. They show that resource use overlap between vertebrates and insects can be intense enough, at least locally, to suggest mutual effects and co-evolutionary responses. Sikes (1996) showed that unidentified vertebrate scavengers were one of the main competitors of the burying beetle, *Nicrophorus nigrita*, for mouse carcasses in California. Drew (1987) found that fruit-eating birds and mammals acted as major natural enemies of fruit-mining insect larvae. In the tropical rain-forest habitat examined, up to 100% of fruits (*Planchonella australis*) were infested by tephritid fruit flies. Frugivorous birds and rodents removed up to 66% of fruits thus controlling insect populations to a similar extent both by reducing the available breeding sites and by ingesting infested fruits. Bigler and Delucchi (1981) similarly found that in a study of tephritid flies breeding in wild olives, frugivorous birds were one of the most important factors causing prepupal mortality. Scott and Black (1981) determined that small, localised populations of weevils were controlled by white-tailed cockatoos in forest ecosystems. Atlegrim (1989) reported a 63% reduction in several insect larvae in bilberries due to indirect bird predation and Zamora and Gomez (1993) found that wild goats in Spain acted as a major predator of a gall-making chalcid species, potentially influencing the spatial distribution of the insects. Halevy (1974) demonstrated that by consuming the pods of various *Acacia* species, gazelles exerted a major mortality force on seed-eating bruchid beetles (see also Lamprey *et al.* 1974). The same was confirmed for bruchid beetles utilizing fruits of a Costa Rican dry forest tree; this time domestic horses and cattle caused high local mortality by eating the fallen fruits on the ground (Herrera 1989). Typical levels for insect-infestation were sometimes estimated: Valburg (1992) found 20% of *Acnistus arborescens* fruits infested, Manzur and Courtney (1984) estimated 10-60% (mean 37%) of infested fruits in their sample of hawthorn (*Crataegus monogyna*) and 23% of bunchberries (*Cornus canadensis*) examined by Burger (1987) contained insects. A few studies suggest that the frequency

of encounters between vertebrates and invertebrates is too low for any important interactions to occur (Traveset 1993) or that vertebrates represent only an 'insignificant mortality factor' (Bateman 1972, Boller and Prokopy 1976, Debouzie 1989); notably all these reports concern tephritid fruit flies, a major agricultural pest of fruits. Although many publications that indicate the degree of resource use overlap involve fruits (or seeds) and insects generally not associated with aggregation, it is not unreasonable to assume similar infestation rates and that the extent of sharing common resources could be equally strong for systems in which aggregation is observed.

Evidence for preference, avoidance or no discrimination

Since most studies have not considered the impact of vertebrate frugivory, granivory or scavenging on insects, it is difficult to generalise and classify the behavioural responses of vertebrates to insect infestation. The majority of studies did not identify the insects that caused infestations, giving only a general indication of invertebrates likely to be present. Broadly, studies could be divided into: (1) direct feeding choice trials under controlled conditions or in the field; (2) field sampling of removal rates (e.g. of rejected fruits); and (3) observational or anecdotal evidence. Feeding choice trials or removal rate measures sometimes tested for preference of infested or non-infested resources, but often they examined other attributes associated with insect infestations (e.g. delay of fruit ripening, general damage to fruits or deformation) or the impact of spoilage by micro-organisms thought to be introduced by insects.

It is well established that birds and mammals use different senses to locate and assess resources: birds have well-developed vision (e.g. Engrizer 1995), even colour vision, and little sense of smell, while many mammals, e.g. bats (Laska 1990) and small mammals (e.g. Fitter 1987), use olfactory cues and have poor vision. Both can also use tactile senses when making judgements about food. Since both olfactory and visual cues may be altered by insect infestation, responses can be expected for both vertebrate groups. Evidence for mammals is summarised in Table 2.1 and for birds in Table 2.2.

Table 1. Mammal responses to invertebrate-infested resources. The table summarises the species of mammal involved, the insect species affected, the feeding response of mammals to infestation; the resource type where interactions occurred and how the response was sampled.

vertebrate species	squirrel (*Sciurus carolinensis*)	grey squirrel (*Sciurus carolinensis*)	bats (*Sturnira ludovici*, *Carollia brevicauda*)	squirrel	white-footed mouse (*Peromyscus leucopus*) short-tailed shrew (*Blarina brevicauda*)
invertebrate species	cecidomyiid fly (*Asphondylia ilicicola*)	curculionid beetle (*Conotrachelus affinis*)	unidentified	*Balaninus* larva *Clisiocampa*	curculionid weevil (*Curculio paradalis Conotrachelus naso*)
response	avoidance	avoidance	avoidance	discrimination only after opening[c]	no discrimination for whole acorns; avoidance of infested nutmeat
resource type	American holly (*Ilex opaca*)	hickory (*Carya glabra*)	*Acnistus arborescens*	oak pitch pine	white oak (*Quercus alba*)
type	field sampling of removal rates; test for ripening[a]	field sampling; test for viable nuts[b]	feeding choice trial; test for infestation	field observation	field sampling and feeding choice trial; test for infestation
reference	Krischik *et al.* 1989	Sork & Boucher 1977	Engriser 1995	Davis 1907	Semel & Andersen 1988

[a] squirrels preferred ripe berries; ripening is delayed by insects
[b] squirrels preferred viable nuts; insect-infested nuts become unviable
[c] insects are prime target

Table 1. (continued)

vertebrate species	grey squirrel (*Sciurus carolinensis*)	Pyrenean ibex (goat) (*Capra pyrenaica*)	gazelle	horse, cattle	house mouse (*Mus musculus*)
invertebrate species	curculionid larva	chalcid gall maker (*Systasis encyrtoides*)	bruchid beetle	bruchid beetle (*Amblycerus cistelinus*)	pea weevil (*Bruchus pisorum*)
response	no discrimination	no discrimination	no discrimination	no discrimination	preference[d]
resource type	acorn (*Quercus nigra*)	mediterrannean shrub (*Hormathopylla spinosa*)	*Acacia* sp.	*Guazuma ulmifolia*	pea
type	feeding choice trial; test for infestation	field sampling of browsing behaviour	field sampling of browsing behaviour	field sampling of browsing behaviour	field observation
reference	Weckerly *et al.* 1989	Zamora & Gormez 1993	Lamprey *et al.* 1974; Halevy 1974	Herrera 1989	Lindusky 1942

[d] insects are prime target

Table 1. (continued)

vertebrate species	white footed mouse (*Peromyscus leucopos*)	rodent species[f] (*Clethrionomys glareolus* and *Apodemus sylvaticus*)	marmoset (*Callithrix jacchus penicilata*)	mangabey (*Cercocebus albigena*)
invertebrate species	tephritid fly[e] (*Rhagoletis cornivora*)	tephritid fly (*Phagocarpus permundus*) lepidopteran larvae (*Blastocacna helerella*)	*Tenebrio* larva	unidentified fig-mining insects
response	preference	preference	preference	preference[g]
resource type	silky dogwood (*Cornus amomum*)	hawthorn (*Crataegus monogyna*)	banana	fig (*Ficus natalensis* and *Ficus vallis-choudae*)
type	feeding choice trial; test for damaged, rotting fruits	field observation	feeding choice trial; test for infestation	field observation
reference	Borowicz 1988	Manzur & Courtney 1984	Redford *et al.* 1984	Freeland 1979

[e] generally known to oviposit in fruit type; not identified specifically in this study
[f] generally known to consume hawthorn in area; not indentified specifically in this study
[g] insects are prime target

Table 2. Bird responses to invertebrate-infested resources (see Table 1 for further details)

vertebrate species	northern mockingbird (*Mimus polyglottos*) grey catbird (*Dumetella carolinensis*) white-throated sparrow (*Zonotricia albicollis*)	passerine birds including; mockingbird, bluejay, starling, cardinal, robin, wood thrush	passerine birds including: American robin, hermit thrush, white-throated sparrow	blackbird (*Turdus merula*) song thrush (*Turdus philomelos*) silvereye (*Zosterops lateralis*) kereru (*Hemiphaga novaeseelandiae*)
invertebrate species	thephritid fly[a] (*Rhagoletis cornivora*)	cecidomyiid fly (*Asphondylia ilicicola*)	unidentified invertebrates	unidentified
response	avoidance	avoidance	avoidance	avoidance
resource type	silky dogwood (*Cornus amomum*)	American holly (*Ilex opaca*)	bunchberry (*Cornus canadensis*)	Darwin's barberry (*Berberis darwinii*)
type	feeding choice trial; test for damaged, rotting fruits	field sampling of removal rates; test for ripening[b]	field sampling of removal rates; test for damaged fruits	feeding choice trial; test for damaged fruits
reference	Borowicz 1988	Krischik *et al.* 1989; Kreuger & Potter 1994	Burger 1987	Allen & Lee 1992

[a] generally known to oviposit in fruit type; not identified specifically in this study
[b] birds preferred ripe berries; ripening is delayed by insects

Table 2. (continued)

vertebrate species	blue jay (*Cyanocitta cristata*)	passerine birds including; Clark's nutcracker, Pinyon jay	cedar waxwing (*Bombycilla cedrorum*)	blackbird[1] (*Turdus merula*) unidentified tit species[2]
invertebrate species	curculionid weevil	*Dioryctria albovitella*[c]		tephritid fly (*Phagocarpus permundus*) lepidopteran larvae (*Blastodacna helerella*)
response	avoidance	avoidance	avoidance	avoidance[1] preference[2]
resource type	pin oak (*Quercus palustris*)	pinyon pine (*Pinus edulis*)	*Ilex opaca* *Lonicera maackii maxim* *Prunus caroliniana* *Phytolacca americana* *Cornus florida*	hawthorn (*Crataegus monogyna*)
type	feeding choice trial; test for infestation	field sampling of removal rates	feeding choice trial; test for microbially infected fruits[d]	field sampling of rejected fruits
reference	Dixon *et al.* 1997	Christensen & Whitham 1991	Buchholz & Levey 1990	Manzur & Courtney 1984

[c] generally known herbivorous 'predators' of pine cones; not indentified specifically in study
[d] microbial infection often facitlitated by insect infestation

Table 2. (continued)

vertebrate species	common bush-tanager (*Chlorospingus ophthalmicus*)	unidentified bird species	thrush[1] (*Turdus migratorius*) American robin[2] (*Ixoreus naevitus*)	white-tailed black cockatoo (*Claryptorhynchus funereus latirostris*)
invertebrate species	unidentified pulp-mining larvae	chalcidoid wasp species (*Sycophila* sp., *Megastigmus pistaceae*, *Cyrtoptix* sp., *Eurytoma* sp.)	sawfly[1] (*Melastola resinicolor*) lepidoptera[f] (*Lotisma trigonana*)	weevil (*Alphitopis nevea*)
response	avoidance[1] no discrimination[2] preference[3]	no effect[e]	no discrimination[1] preference[2]	no discrimination[g]
resource type	various shrubs: *Nea ampifolia*[1] *Lysianthes synanthera*[1] *Solanum cordovense*[2] *Acnistus arborescens*[3] *Cestrum racemosa*[3] *Gonzalagunia rosea*[3] *Ardisia compressa*[3]	*Pistacia terebinthus*	early blueberry (*Vaccinium ovalifolium*)	*Banksia attenuata*
type	feeding choice trial; test for infestation and damaged fruits	field sampling of removal rates and feeding choice trial	feeding choice trial and field sampling of removal rates; test for deformed fruits	field sampling of removal rates; test for infestation
reference	Valburg 1992	Traveset 1993	Traveset *et al.* 1995	Scott & Black 1981

[e] resource overlap is deemed too small for any systematic effect
[f] generally known to infest berries; not identified specifically in this study
[g] insects are prime target

Mammals

Evidence from mammals showed great variation between studies even when considering the same species. Grey squirrels (*Sciurus carolinensis*), for example, avoided infested nuts or fruits (Sork and Boucher 1977, Krischik *et al.* 1989) but sometimes only after opening the shell of the nut (Davis 1907) while sometimes they did not discriminate at all (Weckerly *et al.* 1989). Inconsistent results may be due to the varying types of resources used and they highlight that it is often impossible to generalize responses into categories. All too often, the behaviour is likely to vary, for example, with the food type, the infesting insects, the duration of the infestation or nutritional status of the vertebrate. Two species of fruit bats in Engrizer's (1995) study avoided infested fruits but generally most mammals were either indiscriminate about infestation, like large herbivores feeding on fruits of various tree and shrub species (Lamprey *et al.* 1974, Herrera 1989, Zamora and Gomez 1993), or preferred infested resources. Preference for infested resources was seen in rodents other than squirrels (Lindusky 1942, Manzur and Courtney 1984, Borowicz 1988) and primates (Freeland 1979, Redford *et al.* 1984). Interestingly, the infested resources preferred by primates consisted of figs and bananas, fruits commonly used by aggregating flies of the genus *Drosophila*. Some mammals searched specifically for the insects although initially the feeding response was thought to be frugivory or granivory (Davis 1907, Lindusky 1942, Freeland 1979). Redford *et al.* (1984) suggest that what is often termed frugivory in primates may, in fact, be insectivorous behaviour.

Birds

In birds, the number of studies in which insect-infested or insect-damaged fruits and seeds were rejected clearly dominated, although in some cases, the exact response varied with resource type (e.g. Valburg 1992) and the species of bird (Manzur and Courtney 1984, Traveset *et al.* 1995). The high rejection rate of infested resources is somewhat surprising. Many birds include insects in their diet, especially in the breeding season when the dietary requirements of chicks have to be satisfied (e.g. Motis *et al.* 1997). Even predominantly frugivorous birds feed on animal prey, probably to overcome nitrogen deficiencies in fruit pulp (e.g. Jordano and Herrera 1981) and are thus not strictly frugivorous. Yet, it appears that when looking for fruits, most birds are adverse to insect infestation. The discrepancy may be explained because birds are responding to the insect-induced changes to fruits (colour, taste, smell) or because the insects infesting resources differ from those that are preyed upon. European starlings

(*Sturnus vulgaris*), for example, are known to take mainly larger invertebrate prey, e.g. chrysomelid beetles, lepidopteran larvae, araneids or flower flies (Syrphidae) (Motis *et al.* 1997) while house sparrows (*Passer domesticus*) prefer lepidopteran larvae longer than 5 mm (Madej and Clay 1991).

Summarising vertebrate choices

Feeding decision cues and responses vary substantially between different taxa of vertebrates that are attracted to resources shared with insects. More birds than mammals avoided insect infested resources but it was obviously impossible to evaluate the frequency of encounters between vertebrates and invertebrates in each example. More studies investigating this link are essential to assess the likelihood of vertebrates' responses affecting insect behaviour on an evolutionary scale. From some studies however, it appears that the behaviour displayed by vertebrates could indeed affect insect populations, at least at a local level. It remains difficult to generalize; local community composition and population densities as well as resource abundance are likely to be determining factors. While there is clearly a potential for aggregates of insects to deter many bird and some mammal species, studies involving resources and insects where aggregation is the norm are still lacking. It has yet to be determined whether vertebrates only respond to the presence or absence of insects in resources or whether they can also discriminate between different densities of insects.

Can vertebrates detect densities of insect-infestation in fruits?

Methods

1) Two species of small mammals, wood mouse (*Apodemus sylvaticus*) and bank vole (*Clethrionomys glareolus*) were trapped over night along hedgerows and in small woodlands near York, England, from November 1996 to February 1997. Animals were caged individually and kept in the laboratory for approximately eight hours on a diet of dried hamster food with access to water. A feeding choice trial involved exposure to two even-sized pieces of apple (range 3.85 to 9.97 g),either non-infested or infested with varying densities of second and third instar larvae of the genus *Drosophila*. *Drosophila* species used were *D. simulans*, *D. subobscura* and *D. funebris*; the latter two are commonly found near habitation and in woodlands in Britain and are known to use

Table 2.3. Results of Analysis of Covariance on the proportion of apple eaten that was infested for the effects of mammal species (wood mouse, bank vole), *Drosophila* species (*D. simulans*, *D. subobscura*, *D. funebris*), larval infestation time (1, 5 days) and larval densities (covariate). ns = non-significant.

Source	SS	d.f.	F-ratio
Covariate			
larval density	0.011	1	0.908 ns
Main effects			
mammal (M)	0.005	1	0.408 ns
Drosophila (D)	0.032	2	1.302 ns
infestation time (T)	0.009	1	0.688 ns
2-way interactions			
M x D	0.007	2	0.287 ns
M x T	0.001	1	0.084 ns
D x T	0.001	2	0.035 ns
3-way interaction			
M x D x T	0.063	2	2.535 ns
error	0.586	47	

Table 2.4. Mean percentages (± SD) of apple eaten of each class of larval infestation density for feeding trials excluding and including starlings. N = number of trials.

Density class	N	Mean %	SD
excluding starlings			
none	3	25.03	39.11
medium	3	4.35	23.88
high	3	0.40	2.83
including starlings			
none	5	56.49	10.24
medium	5	51.96	5.18
high	5	57.83	0.76

resources consumed by both mammal species. Infestation time was either 1 or 5 days and non-infested resources were aged for the same length of time. Larval densities ranged from 40 to 100 larvae per piece of apple, i.e. 4.67 to 31.11 larvae per g fresh weight. Experiments were conducted under controlled conditions in the laboratory, mammals were not food deprived but had continuous access to dried hamster food.. Fruits were weighed before and after each 16 hour trial period. Each mammal was used only once and the trials were replicated five times for each mammal species, *Drosophila* species and larval mining time combination giving a total of 60 trials.

2) Several common bird species were examined for preference or avoidance of apple infested with varying densities of *D. subobscura* larvae from March to July 1998 using garden feeding stations near Maidstone, Kent, England. Insect densities were fixed at three levels ('none' = 0 larva; 'medium' = 0.5 - 2.0 larvae g^{-1}; 'high' = 6.1-19.8 larvae $^{b-1}$) and three apple segments, one of each level, were presented simultaneously to birds on feeding platforms. Segments were weighed before and after each 2 hour trial (8 trials in total). Additionally, the species of birds predominantly using the station during this time were noted.

Results and Discussion

1) Although apple segments containing high densities of larvae showed far more signs of rot (and sometimes mould), neither *A. sylvaticus* nor *C. glareolus* displayed any preference for infested or non-infested fruit. This was not influenced by the species of *Drosophila*, infestation time or larval density (Table 2.3). The ratio of infested over non-infested fruit eaten was close to one although wood mice consumed slightly more infested (1.11 / 1) while the reverse was true for bank voles (0.87 / 1). Both small mammals are known to feed on flesh and seeds of many fruits and to include invertebrates into their diet although the degree of carnivory is lower in bank voles than in wood mice (Watts 1968, Eldridge 1969, Montgomery and Montgomery 1990, Castien and Gosalbez 1996). While it is possible that larvae were simply not detected it is also clear that symptoms associated with *Drosophila* infestation (e.g. fermentation due to presence of yeasts) did not influence food choice in either *A. sylvaticus* or *C. glareolus*.

composition could be controlled for, results were very variable. Trials could be divided into those in which mainly sparrows (*Passer domesticus*) and chaffinches (*Fringilla coelebs*) frequented the feeding station and those where starlings (*Sturnus vulgaris*) visited. Such division is reasonable because sparrows and chaffinches are predominantly seed eaters (Hollom 1962, Filix 1977) while starlings are known for their high level of omnivory (Feare 1985). In the trials, starlings did not respond to presence, absence or the density of *Drosophila* larvae whereas sparrows and chaffinches consumed less of apple from the 'medium' infestation compared to 'none' and hardly any from the 'high' infestation (Table 2.4).

The results conform to the general pattern that birds rather than mammals are adverse to the presence of invertebrates in the resources they consume. There is some indication that birds may even respond to densities of insects although it is unclear whether they react to the actual larval numbers or to the insect-induced changes in fruits which alter with different densities. It is important to note that the sample sizes for bird trials were very small while the experimental conditions were highly uncontrolled. A lot more replicates would be needed for these statements to be made with any real confidence,. yet, at least the tendency is there. While the response itself and the mechanisms underlying it have yet to be examined more closely, this study shows that there is nevertheless a potential for aggregation functioning as a strategy to deter, at least, some bird predators. It also highlights that our understanding of the impact of these interactions on insect ecology and evolution is still very limited although it is highly likely that some effects of vertebrates may have been constant enough throughout the evolution of insects for this impact to be of importance (Sallabank and Courtney 1992). Little information is available for insects breeding in carrion or fungi although the frequency of encounters and the number of insect and vertebrate species sharing such resources is likely to be as high and vast as in fruits and seeds. It is not possible to confirm or reject at this stage, whether aggregation might, at least in part, represent a strategy that could avoid the risk of such cryptic predation. Considering the immense number of species (plants, vertebrates and insects) involved in this type of interaction on a global scale, it is surprising that there are still so many unanswered questions.

Chapter 3 - Allee effects in *D. simulans*

Summary

The occurrence of Allee effects in *D. simulans* was investigated on grapes and banana under different treatment conditions, in particular yeast preparations. Larval densities either represented female oviposition choices (grapes) or were manipulated (banana). Survivorship and adult body size (thorax lengths) showed some Allee effects but these were significant only when larval densities were experimentally controlled and depended highly on the precise treatment conditions. The growth of mould proved unlikely to be a driving factor for Allee effects while interactions between yeast and larval densities indicated that competitive effects may be responsible for the occurrence of Allee effects in *D. simulans*. The frequency with which the precise conditions under which Allee effects were observed would be encountered in the field is unknown, but it is unlikely that Allee effects are very important in the evolution of aggregation.

Introduction

Allee (1931) first described the observation that for many organisms, population growth is maximised 'at intermediate population densities rather than with too few or too many [individuals] present'. This observation is now commonly referred to as the Allee effect and is generally used to describe the decrease in the net recruitment rate experienced at population densities not only above but also below a certain optimum. Allee effects are often reported in studies of mate finding, e.g. in parasitoid wasps (Fauvergue *et al.* 1995) or sheep ticks (Andrewartha & Birch 1954) where breeding is inhibited by the low density of individuals. Other examples of Allee effects in insect populations can be seen in bees as a results of thermo-regulation requirements (Winston 1987) or in conifer sawflies through group defence (Codella & Raffa 1995).

Importantly, Allee effects can also occur through a lack of co-operation between conspecifics where co-operation is required to modify the environment in some way. For the grain borer *Rhizopertha dominica*, a sufficiently large number of individuals is needed to damage the grains for oviposition and larval development (Crombie 1944). Similar intra-specific facilitation has also been observed in *Drosophila*. Both survival and body size or weight in *Drosophila* are highly dependent on larval rearing densities and decrease with increasing competition from conspecifics (e.g. Atkinson 1979;

Calgari 1980; Grimaldi & Jaenike 1984). Allee himself (1938) noted however, that the highest numbers of *Drosophila* were produced when the feeding surface in culture vials relative to larval density was neither too great nor too small. He attributed the observation to the growth of wild yeasts or mould which developed most rapidly when not controlled by sufficient numbers of larvae, feeding on and churning up the surface of the medium. Subsequently, a similar effect has been noticed by a number of authors. Lewontin (1955) transferred larvae of *D. melanogaster* at different densities to vials containing yeasted culture medium. Although it is not clear whether the effect was significant, he demonstrated that the optimum for producing maximum numbers of emerging adults was at intermediate larval densities. These findings are supported in studies by Sokoloff (1955) for *D. pseudoobscura*, *D. perimilis* and *D. miranda* and by Courtney *et al.* (1990) for the mushroom-breeding *D. suboccidentalis*. In both studies larval growth and development or survival were optimised at intermediate rearing densities although, again it is not clear from the data whether the effects were significant.

The mechanism for the Allee effect in *Drosophila* is generally attributed to the development of mould which may render a patch unsuitable for larvae but larval grazing may control the spread of such moulds (Kearsey 1965; Atkinson 1979; Courtney *et al.* 1990). In addition, the surface churning of many larvae is supposed to facilitate growth of beneficial yeasts on which *Drosophila* larvae feed (e.g. Sang 1950). The Allee effect clearly influences survival and reproduction in small populations both in the field (e.g. Lamont *et al.* 1993) and in model systems (Stephan & Wissel 1994). I argue that the co-occurrence of Allee effects and aggregation may be linked. In *Drosophila* it could represent a selective force for aggregating phenotypes during periods of low local population size, i.e. when the benefits derived through intra-specific facilitation outweigh the effects of intra-specific (or sibling) competition. If fitness was generally enhanced at intermediate densities, a gene or gene complex that promoted aggregation could remain in the population even if the strategy is disadvantageous at higher densities.

Although the Allee effect has been noticed in *Drosophila*, careful manipulation of densities and treatment conditions have not yet been attempted and the extent of the effect has not been quantified. A variety of factors have potentially strong effects on larval development and survival. They include, e.g. temperature, moisture, rearing

density and nutritional quality of the medium (discussed in Sang 1949). Yeasts are one important microbial component of the feeding and breeding sites of *Drosophila* (e.g. Vacek *et al.* 1985). The majority of *Drosophila* species have absolute requirements for yeast both during adult and larval life stages (Kearney & Shorrocks 1981) probably from a lack of ability to synthesise important sterols which in turn are abundant in yeasts. The importance of yeasts is also suggested by studies of cactophilic *Drosophila* (Barker *et al.* 1981) and other *Drosophila* species (e.g. Dobzhansky *et al.* 1956; Ali & El-Helw 1974); these flies are differentially attracted to varying yeast species. Kearney & Shorrocks (1981) demonstrated that larvae do not feed solely on yeast but respond strongly to the yeast-medium complex in terms of survival and development. It is unclear whether this sensitivity is due to the larval requirements for nutrients other than those supplied by yeasts or due to the variable nutritional value of yeasts growing on different media.

The aims in this study were to investigate the effect of larval rearing density on survivorship and body size in laboratory populations of *D. simulans*. In order to gain insight into the conditions that may be required for Allee effects to operate the interaction of density with different fruit types and a range of treatments including different preparations of yeast and inoculation times were compared. Importantly, I present a comparison of conclusions from both a carefully manipulated experiment to one that uses, statistically, more problematic designs.

Materials & Methods

Flies

The stock of flies used during this set of experiments were from a wild strain of *D. simulans* collected in Zimbabwe, Africa. *Drosophila simulans* are tropical in origin but are now distributed very wildly on a global scale (Lemeunier *et al.* 1986). Outside the tropics they are found mainly in many man-made habitats, e.g. orchards (Nunney 1990), fruit markets (Atkinson & Shorrocks, 1977), breweries (Newbury 1984) and vineyards (McKenzie 1974). The stock had been established for about four years and was maintained on standard *Drosophila* medium (see appendix) in 25 mm x 75 mm glass vials.

Allee effect on grapes

The grapes used in these experiments were white, seedless grapes, known to be a suitable oviposition site for *D. simulans* (e.g. Dytham *et al.* 1992). All grapes were frozen on the day of purchase and defrosted thoroughly for 4 hours before used. Grapes were used untreated ('plain'), or treated with active 1% w/v baker's yeast solution (*Saccharomyces cerevisiae*). Treatment consisted of either soaking the halves for 7 days in the yeast solution ('soaked') or briefly dipping them into it prior to use ('dipped'). For each experimental batch all grapes originated from the same bunch of grapes; different bunches were used across the experiment.

Female *D. simulans* were allowed to oviposit individually for 24 hours on four grape halves. Thus neither egg nor larval densities were controlled or manipulated which are the conditions under which Allee effects have commonly been noticed. The number of eggs on each grape was established exactly using a binocular microscope; grapes containing no eggs were discarded. The remaining halves were incubated individually in 25 mm x 75 mm glass vials stoppered with cotton wool at 22° ± 1°C. The grapes were re-examined after 48 hours to record the number of hatched larvae, non-viable eggs were ignored in the following analysis. Grapes were then checked every 4-5 days for emergent adults which were isolated and stored in 70% ethanol. Two fitness parameters were established: survivorship (proportion of original number of larvae per grape that eclosed) and size (wing and thorax length). Thoraxes were measured from the midpoint of the anterior margin of the mesothorax to the distal midpoint of the scutellum. Wing lengths were measured from the anterior cross vein to the distal end of the 3rd longitudinal vein. Both measures are reliable indicators of body size used in many previous studies (e.g. Robertson 1956; Pitnick 1991). Since *Drosophila* are sexually dimorphic, the sex of the emergent adults was also recorded. A (crude) measure of the mould present on each grape half was made by estimating the percentage cover of the half during the regular examinations. The level of replication was as follows:

plain grapes 288 halves (11 batches)
yeast-soaked 58 halves (2 batches)
yeast-dipped 137 halves (4 batches)

Allee effect on banana

To contrast the results of the relatively uncontrolled set-up described above I designed an experiment where the number of larvae present on a unit resource was carefully manipulated and balanced in terms of density classes in a fully factorial design. Prior to each trial, egg collecting plates were prepared. These consisted of a 90 mm petri dish, half filled with 1% agar gel. The gel was covered with a thin layer of 1% baker's yeast solution which was either active or heat-killed through microwave treatment. Between 50-100 flies were allowed to lay eggs for about 24 hours after which early emerging first-instar larvae were collected. Ripe bananas were mashed and were either used fresh or covered and left to age for one week in the laboratory. Ageing the medium was thought to increase the chance of contamination with fungi and bacteria. Larvae were transferred onto 0.52 g of mashed banana (the mean weight resulting from filling the lids of 50 x 13 mm glass specimen tubes to the level of the rim) in densities of 1, 2, 5 and 10 larvae per quantity of medium. The only yeast deliberately added to the medium thus originated from the transferral of larvae from collection plates. The different yeast and banana treatments resulted in four possible combinations with the following replication levels:

1. fresh banana; active yeast (5 batches)
2. fresh banana; heat-killed yeast (5 batches)
3. aged banana; active yeast (10 batches)
4. aged banana; heat-killed yeast (5 batches)

A batch consisted of the four density classes, replicated to equalise the number of larvae per class in each trial:

number of larvae per 0.52 g banana	replicates
1	10
2	5
5	2
10	1

Overall, 25 batches were prepared giving a total of 250 larvae per density class (1000 in total). All larvae were placed in an incubator at 22° ± 1°C and checked every 4-5 days for emerging adult flies. The final parameters recorded consisted of survivorship (proportion of flies eclosing from 10 larvae transferred per density class in a batch) and size (see above). As the surface of banana medium was very small, a reliable estimate of mould cover was difficult. Instead I scored simply for presence or absence of mould.

Allee effects in the F2 generation

As it is possible that Allee effects may express themselves in a product of F1 survival and body size-related changes in fecundity, I followed the impact of any effects on survival and size into the next (F2) generation. Size shows a positive (genetic) relationship to reproductive success in female *Drosophila melanogaster* (Sang 1950; Robertson 1957; Partridge *et al.* 1986) and has been linked to higher fitness in males, through an increase in mating success (Pitnick 1991, Partridge & Fowler 1993). It is possible then, to predict adult lifetime progeny production for a given body size measurement (usually thorax length). I calculated the expected progeny for female *D. sim ulans*, using a regression line from Partridge *et al.* (1986) for female *D. melanogaster*. These species are closely related and I assumed that the essential aspect of the relationship would be similar. For males, no such line could be found but I re-plotted the data from Partridge (1988) for male *D. melanogaster* and obtained a significant linear relationship which was used in the calcultations. Mean thorax lengths for each density class in each treatment combination were used. The corresponding male and female reproductive output (RO) can be used together with the mean survivorship results (F1 generation) to calculate the expected F2 for different densities and across the different treatments as:

$$expected\ F2 = (RO_{male} \times 0.5^{a'} \times survival + RO_{female} \times 0.5^{a'} \times survival)$$

[a'] the sex ratio did not differ significantly from 1:1 in any of the treatments

Statistical Analysis

Analysis of body size data was straightforward as neither thorax nor wing size measurements deviated significantly from a normal distribution. On grapes, larval numbers were pooled into classes to facilitate analyses of the relationships which were anticipated to be non-linear. Five equal-sized groups (based on percentiles) were established; group 1 was split further to investigate more closely the response of fitness parameters to small changes at low densities. Classes were: 1 (1-2 larvae per grape half), 2 (3-8), 3 (9-16), 4 (17-24), 5 (25-38) and 6 (39+); I subsequently refer to these classes as densities rather than numbers. The difference between results obtained for densities (larvae per g fresh weight) and numbers (larvae per unit resource) was however, investigated.

On banana, further pooling was not necessary as classes had been predetermined (both of numbers and densities). I used two-way and three-way analysis of variance

Table 3.1. Mean survivorship (to emergence) and standard deviation for larvae on different fruit types and for different treatments and levels of replication. N on banana medium = number of replicated density classes in sample; N on grapes = number of grape halves in sample.

Resource	N	Mean Survival	SD
Grape			
plain	288	0.19	0.04
soaked	58	0.27	0.08
dipped	137	0.45	0.05
Banana			
fresh/killed yeast	18	0.71	0.16
aged/killed yeast	38	0.65	0.15
aged/active yeast	19	0.62	0.15
fresh/active yeast	19	0.60	0.21

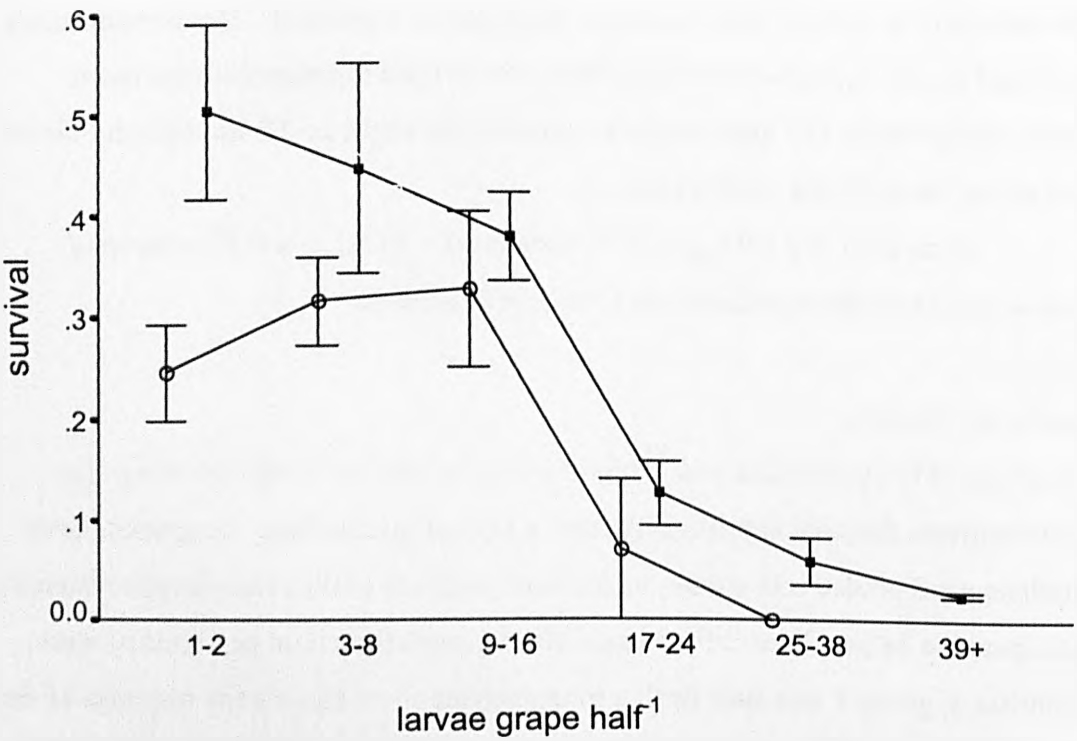

Fig. 3.1. Survivorship (mean proportion ± 1 s.e.) in response to different larval rearing densities. Open circles = plain grapes; solid squares = soaked grapes. The graph for survival on dipped halves is omitted as the response was non-significant.

(ANOVA) to factor out the effects of treatment and density. Least significant difference (LSD) tests were applied to means, where applicable, to analyse responses more closely. The survival data are more difficult to treat. If, as on the grapes, the larval numbers per unit resource are not controlled and balanced by the experimenter, the resulting distributions of survivorship are generally not normal. Larval numbers of 1, for example, will result in survival proportions of either 0 or 1. The generated distribution of survival proportions then will have too many observations in the tail ends depending on the frequency of low larval numbers in the original sample; a problem which is difficult to overcome by meaningful transformations. Hence, I analysed these data using distribution-free methods, loosing the power of factorial ANOVA. I applied Kruskal Wallis (KW) and Wilcoxon-Mann Whitney tests (WMW) for pairwise comparisons to factor out treatment effects. In the experiments using banana, replication ensured that this was not a problem and the survivorship data conformed to normal distributions, allowing factorial analyses.

To analyse the relationship between mould cover and the two fitness parameters on grapes, percentage cover was divided into classes ranging from 0 (0%), 1 (1-5%), 2 (10-70%) to 3 (80-100%). KW was used to estimate the effect of mould class on survival, three-way ANOVA was applied to test the effect on size for both sexes. On banana, the presence/absence of mould in response to treatment was analysed using Chi^2 test for data arranged in 2 x 2 contingency tables.

Results

Grape weight ranged from 0.76 to 3.51g with a mean of 2.12 g (standard deviation = 0.52). Larval numbers ranged from 1 to 56 per grape half, giving a range of larval densities from 0.32 to 34.8 larvae per g fresh weight of grape. Survivorship was significantly higher on banana medium than on grapes (WMW; $Z = - 8.754$; $p < 0.001$). This effect was not simply due to the much larger range of larval numbers per patch on the grapes (1-56 versus 1-10 on banana) but was still highly significant when comparing equivalent larval densities (larvae g^{-1} of respective medium). Females were significantly larger than males averaging 1.46 mm (range 0.73 mm) compared to 1.29 mm (range 0.77) for wings and 1.00 mm (range 0.46) to 0.88 mm (range 0.41) for thorax lengths. Neither measurement differed significantly between the fruit types for either male or female flies emerging. Wing and thorax lengths correlated very closely

Table 3.2. Results of Kruskal Wallis One-way Analysis of Variance survivorship in response to larval rearing density on grapes.

Treatment	d.f.	X^2	p
plain	4	9.34	0.053
soaked	5	28.45	< 0.001
dipped	5	1.90	0.863

Table 3.3. Mean (+SD) thorax lengths (mm) and sample sizes for female and male *D. simulans* across the different grape treatments.

Treatment	female			male		
	N	Mean	SD	N	Mean	SD
plain	169	1.00	0.06	157	0.87	0.06
soaked	78	0.93	0.06	64	0.83	0.05
dipped	455	1.01	0.05	426	0.89	0.05

Table 3.4. 2-way crossed ANOVA for the effects of treatment (yeast; plain, soaked, dipped) and density class on thorax lengths of female *D. simulans* reared on grapes.

Source	SS	d.f.	F	p
treatment (T)	0.188	2	13.090	< 0.001
density (D)	0.132	4	4.605	0.001
T x D	0.212	8	3.696	< 0.001
residual	4.929	687		

Table 3.5. 2-way crossed ANOVA for the effects of treatment (see above) on thorax lengths of male *D. simulans* reared on grapes.

Source	SS	d.f.	F	p
treatment (T)	0.352	2	27.995	< 0.001
density (D)	0.081	4	3.229	0.012
T x D	0.132	8	2.622	0.008
residual	3.974	632		

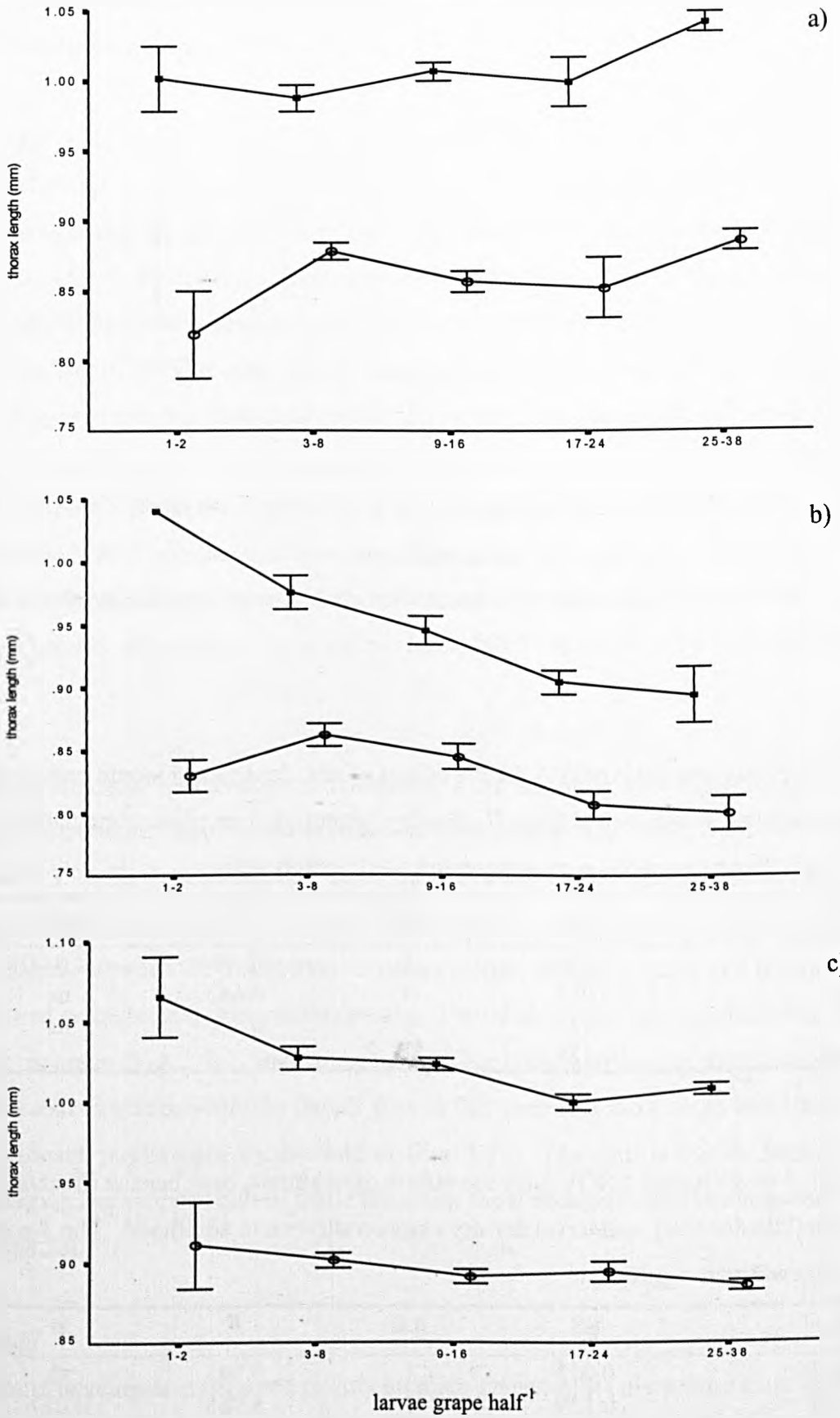

Fig.3.2. Thorax length (mean ± 1 s.e.) in response to initial larval rearing density on grapes. Solid squares = females, open circles = males; a) plain; b) soaked; c) dipped grapes

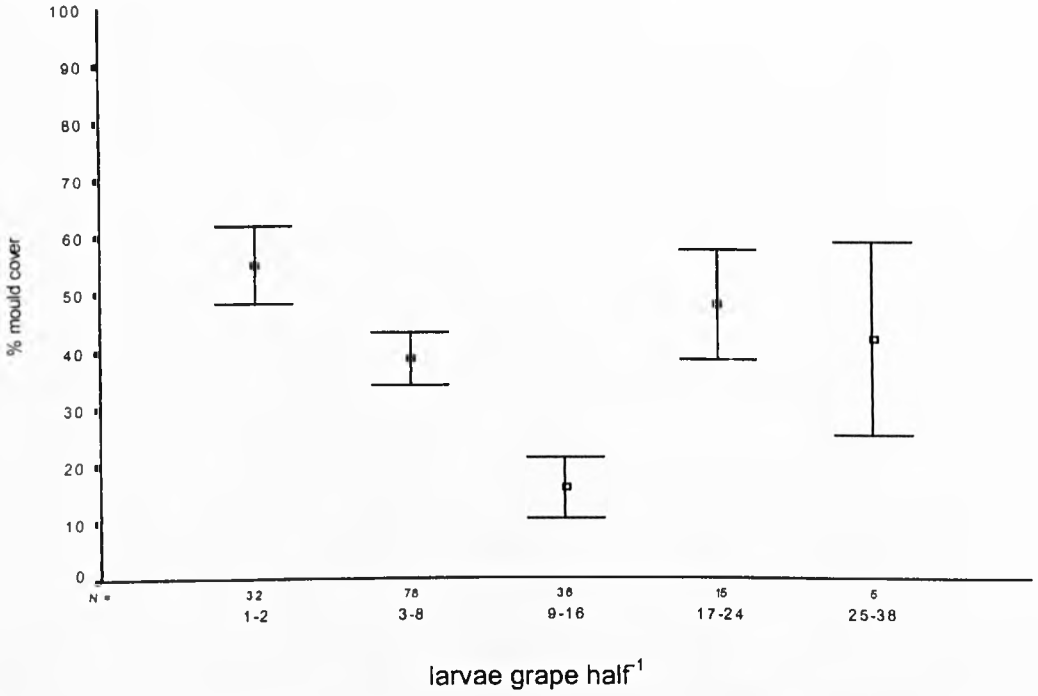

Fig. 3.3. Mean (± 1 s.e.) mould cover (%) on plain grapes in response to initial larval rearing density. KW; $\chi^2 = 18.94$, d.f. = 4, $p < 0.001$.

Table 3.6. 3-way crossed ANOVA for the effects of sex, density and mould cover class on thorax lengths of female and male *D. simulans* emerging from plain grape halves. The higher order interactions were suppressed due to empty cells.

Source	SS	d.f.	F	p
sex	0.414	1	62.944	$p < 0.001$
mould	0.013	3	0.643	ns
density	0.023	4	0.866	ns
residual	0.526	80		

Table 3.7. 3-way crossed ANOVA for the effects of treatment, both banana (fresh/aged) and yeast (killed/active), and larval density class on survival to adulthood. The 3-way interaction was non-significant.

Source	SS	d.f.	F	p
banana (B)	0.013	1	0.543	ns
yeast (Y)	0.139	1	5.965	0.017
larval density (D)	0.400	3	5.708	0.001
(B) x (D)	0.020	1	0.852	ns
(B) x (Y)	0.005	3	0.072	ns
(Y) x (D)	0.332	3	4.736	0.004
residual	1.824	78		

(Pearson's correlation coefficient = 0.932; $p < 0.001$); subsequently I restricted the body size analyses to thorax lengths only.

Grapes

survivorship

Survivorship on grape halves differed significantly between treatments (KW; $X^2 = 88.63$, d.f. = 2, $p < 0001$). Larvae showed higher survivorship on yeasted grapes with the largest proportion emerging from the dipped halves (Table 3.1).

The effect of larval rearing density varied between treatments; there was no response on dipped grapes but on soaked halves survivorship decreased with increasing density (Fig. 3.1, Table 3.2). On plain grapes, the effect was just above the 5% significance level (Table 3.2) but the response seen is one expected for an Allee effect (Fig. 3.1). Pairwise WMW comparisons however, showed that the increase in survival from class 1 to 3 is non-significant. In both plain and soaked treatment categories, survival decreases most notably after class 3 (16 larvae per half).

size

Body size, too, varied between treatments. Flies emerging from dipped grapes were the largest while those from soaked halves were smaller than adults from plain grapes (Table 3.3). Both treatment and initial larval rearing density had significant effects on size (Tables 3.4 and 3.5). The significant interaction terms indicate a different response to density between the treatments. On plain grapes, emerging males and females showed an increase in size with increasing density although this response is significant only in males (Fig. 3.2a). Indication for an Allee effect can be seen for males emerging from soaked grapes while the female flies in this treatment show clear, and highly significant, positive density dependence (Fig. 3.2b). The same is true for female flies emerging from dipped halves while the males show no significant response at all (Fig.3.2c).

mould

Mould development occurred mainly on plain grapes with only some mould found on soaked but not on dipped halves. The effect of mould class on survivorship was significant (KW; $X^2 = 13.767$, d.f. = 3, $p = 0.003$) but this was driven entirely by the very low survivorship in the highest class; the only, but highly significant, pairwise WMW comparison was between class 0 and 3. Larval density did influence mould

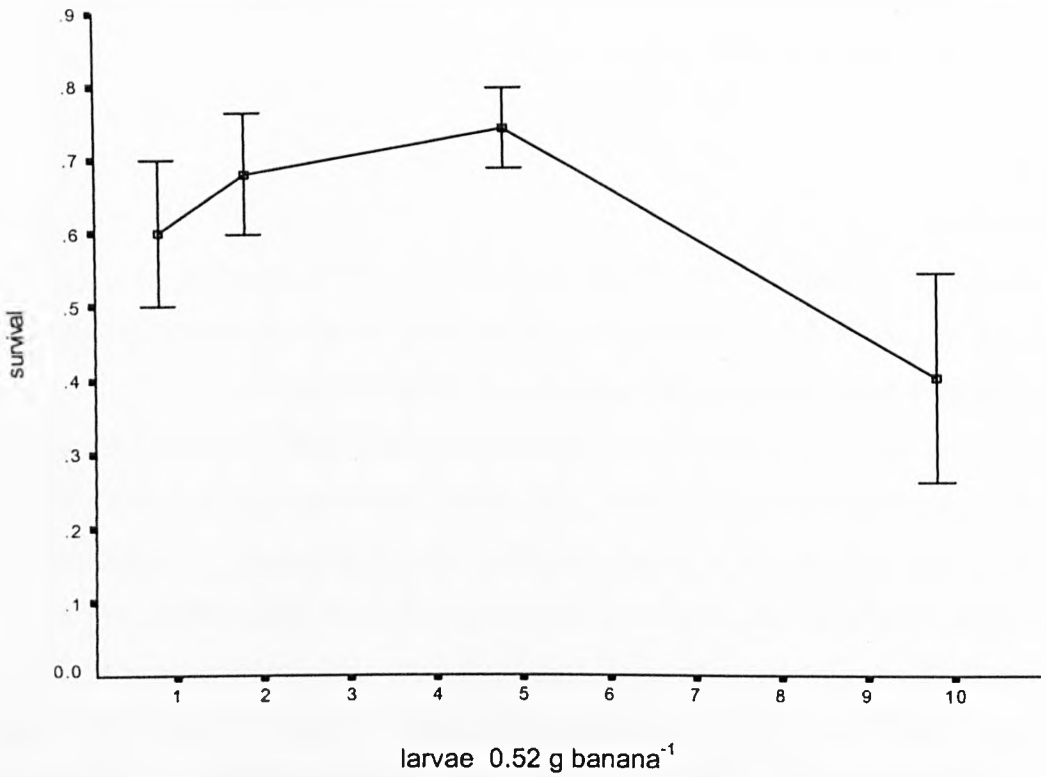

Fig. 3.4. Survivorship to eclosion (mean ± 1 s.e.) of *D. simulans* as a function of initial larval density on aged banana/active yeast medium.

Table 3.8. Mean thorax lengths (mm) and standard deviations for male and female flies across the different treatments.

Treatment	female			male		
	N	Mean	SD	N	Mean	SD
fresh banana/active yeast	56	0.98	0.08	65	0.89	0.06
aged banana/active yeast	68	0.99	0.07	52	0.84	0.06
fresh banana/killed yeast	64	1.01	0.08	70	0.92	0.05
aged banana/killed yeast	136	1.01	0.07	141	0.94	0.09

Table 3.9. 3-way crossed ANOVA for the effects of banana (aged or fresh), yeast (active or killed) and initial larval density (1, 2, 5 or 10) on thorax lengths of female *D. simulans*.

Source	SS	d.f.	F	p
banana (B)	0.00	1	0.09	ns
yeast (Y)	0.04	1	8.07	0.005
larval density (D)	0.21	3	15.55	< 0.001
(B) x (Y)	0.01	1	4.26	ns
(B) x (D)	0.01	3	0.69	ns
(Y) x (D)	0.06	3	4.84	0.003
(B) x (Y) x (D)	0.05	3	3.99	0.008
residual	1.35	308		

cover significantly (KW; $X^2 = 18.94$, d.f. = 4, $p < 0.001$) with a rapid decrease in mould development (≈55 to 15%) from one density class to another (Fig 3.3). At larval numbers higher than 16 per grape half, mould cover increased again. Size was not affected by mould in either sex even when the effects of density were accounted for (Table 3.6).

batch effects

Both survivorship and size were sensitive to the variation in grapes between batches. For both plain and dipped grapes, batch significantly influenced survival (KW; $X^2 = 68.72$, d.f. = 10, $p < 0.001$ and $X^2 = 17.45$, d.f. = 3, $p < 0.001$ for plain and dipped respectively) while there was no such effect in soaked grapes. The effect of batch on male and female size was highly significant (One-Way ANOVA; $p < 0.001$) in all treatments.

Banana

Due to the balanced design of this experiment it was possible to disentangle the effects of ageing the medium, yeast treatment and density more precisely.

survivorship

Survivorship was significantly higher when larvae came from egg-collecting plates with heat-killed yeast, i.e. without the transference of viable yeast cultures. Ageing of the banana medium had no effect while density did influence survival. The significant interaction term between yeast and larval rearing density (Table 3.7) indicates that survivorship was differently affected by density for the two yeast treatments. While density had no effect in the trials with heat-killed yeast, larvae that came from active yeast plates experienced both negative and positive density dependence (Fig. 3.4). The one-way ANOVA for the effect of density on survivorship was highly significant (ANOVA; SS = 0.553, d.f. = 3, $F = 9.569$, $p < 0.001$); the LSD test showed that larvae at densities of 5 per unit resource had a significantly higher chance of surviving through to emergence than those at 1 while at 10 larvae per unit survivorship was significantly lower than at the two intermediate densities. When comparing the response to density in each treatment combination, I found that while in both active-yeast treatments positive density dependence occurred towards larval numbers of 10, the Allee effect was only significant on the aged banana/active yeast medium.

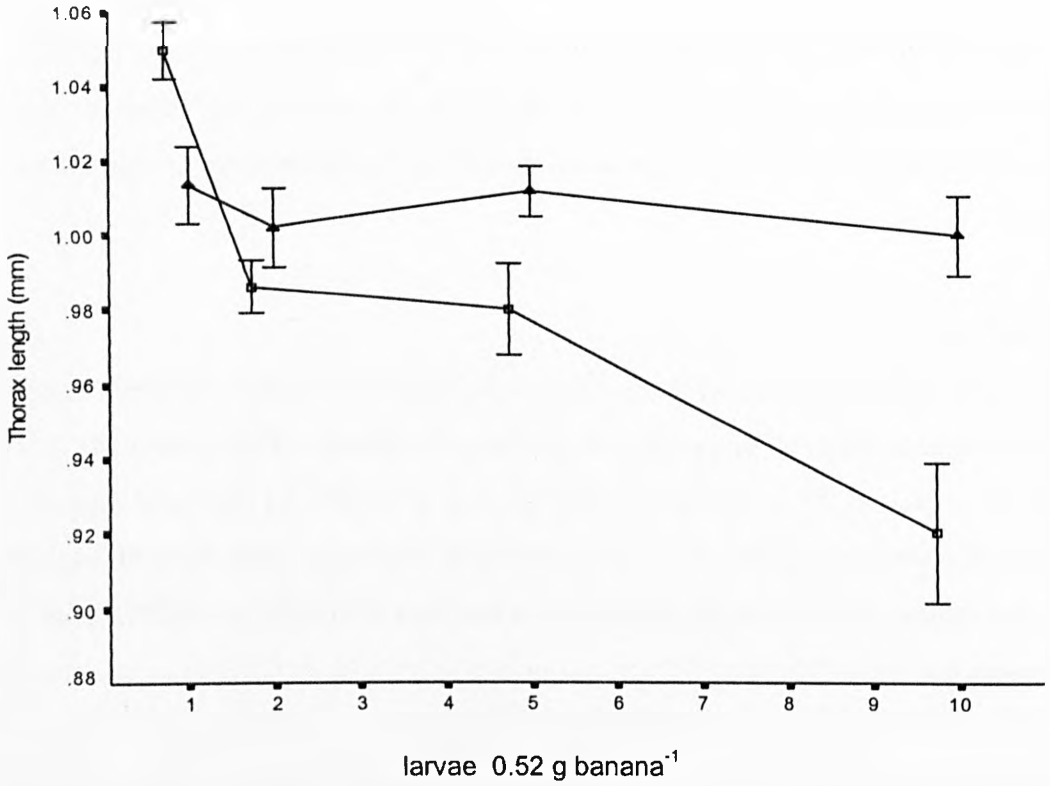

Fig. 3.5. Female thorax length (mean ± 1 s.e.) in response to initial larval rearing density. Solid triangles = killed yeast; open squares = active yeast.

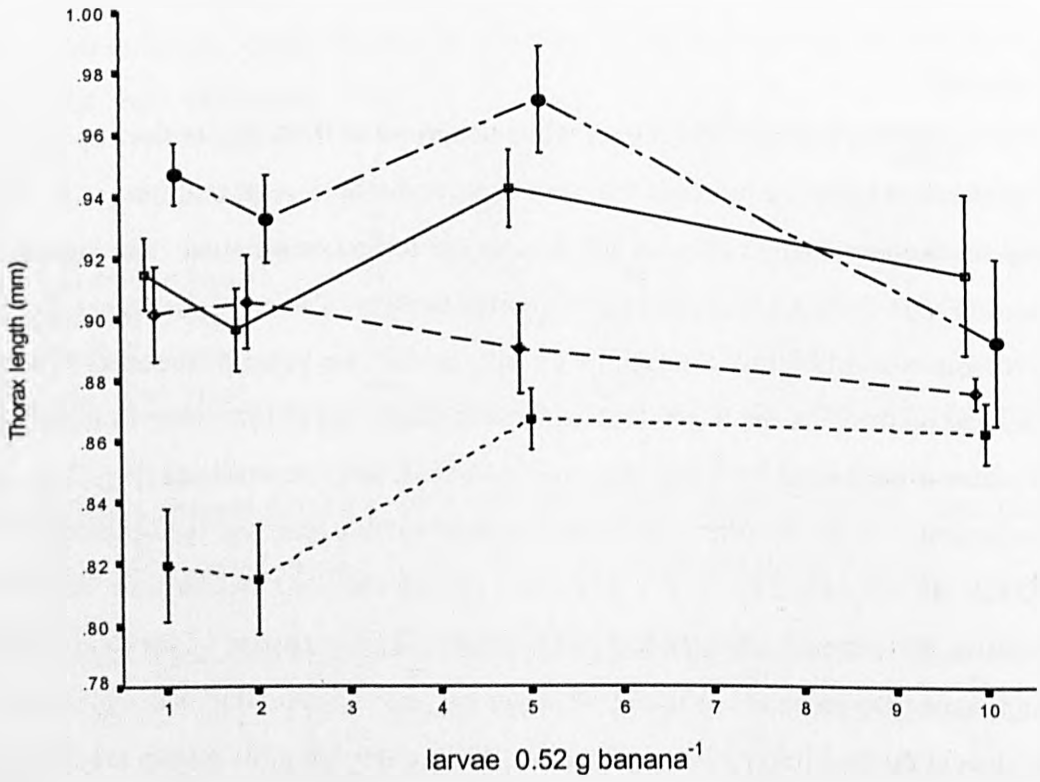

Fig. 3.6. Male thorax (mean ± 1 s.e.) length in response to initial larval rearing density. Solid squares, dotted line = aged banana/killed yeast; solid circles, dot-dashed line = aged banana/active yeast; open squares, solid line = fresh banana/active yeast; open diamond, dashed line = fresh banana/killed yeast

size

The results for size, as on the grapes, were more complex since both sexes responded differently. Mean sizes across treatments are shown in Table 3.8, ANOVA results are given in Tables 9 and 10. Females were significantly smaller when larvae had been transferred from plates with active yeast but were not affected by the ageing of banana. Larval rearing density affected female size in all but one treatment combination hence the significant interaction term for density and yeast (Table 3.9). Where it was significant, size declined with increasing density. Individual one-way ANOVAs for each treatment showed that this response was more marked on active yeast treatments (Fig 3.6). Males were also smaller in trials with active yeast cultures but responded less to larval rearing density; their size was influenced by both treatments, ageing and yeast, indicated by the significant three-way interaction (Table 3.10). There was evidence for an Allee effect on the aged banana/heat-killed yeast treatment with emerging males being significantly smaller at densities of 1 and 2 larvae per unit resource than at 10 and 5 (ANOVA; SS = 0.080, d.f. = 3, F = 3.397, p = 0.01; Fig 3.5). On the aged banana/active yeast medium the decrease in body size with density was without the curve-linear effect; on the other two combinations it was non-significant.

mould

Treatment differentially affected mould occurrence (Table 3.11). Ageing had no effect on absence or presence of mould (Chi^2 goodness of fit to even distribution; $X^2 = 1.904$, d.f. = 1, ns) while active yeast cells significantly inhibited mould development ($X^2 = 173.581$, d.f. = 1, $p < 0.001$; Table 3.11). The effect of density on mould cover was difficult to analyse as there were uneven numbers of receptacles for which presence/absence could be scored in each class. It was observed however, that mould occurred at all density levels.

batch effects

There was no significant difference between batches for survivorship within treatment combinations. For thorax lengths there were some significant effects of batch but p-values were large (One-Way ANOVA; p = 0.042 and p = 0.038 for males and females respectively) relative to those obtained for between treatment or density comparisons.

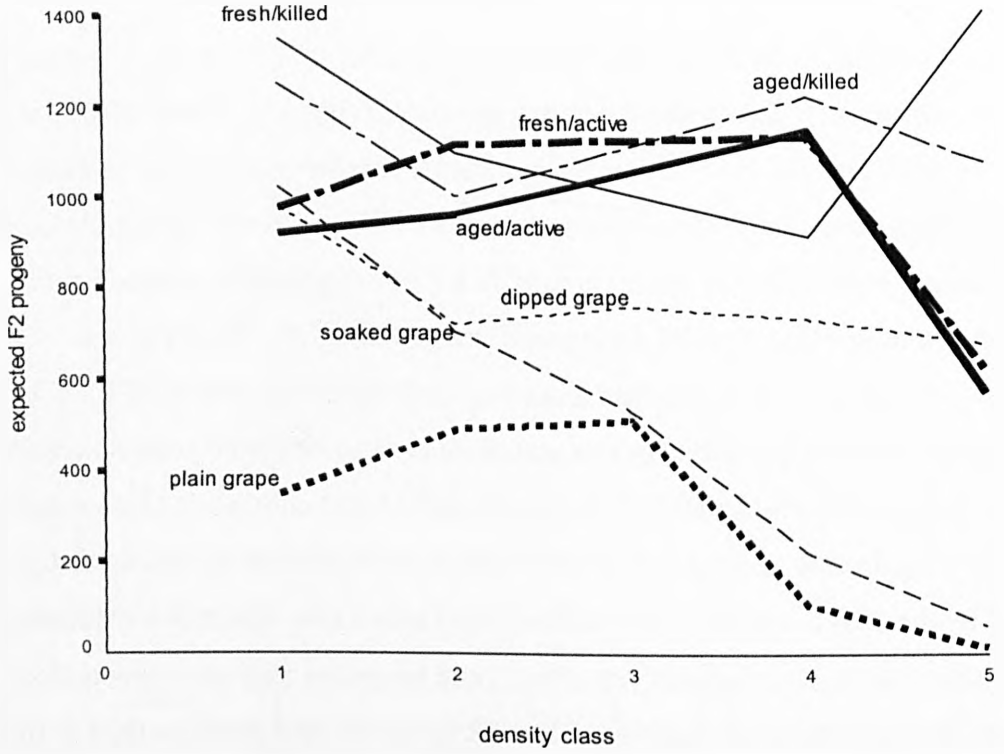

Fig. 3.7. Expected F2 progeny across density classes for the different treatment combinations. Numbers on banana where assigned to classes 1, 2, 4 and 5 as when densities, i.e. larvae per g fresh weight were investigated, this is how they roughly corresponded to the classes on grapes. fresh = fresh banana, aged = aged banana, active = active yeast, killed = killed yeast; bold lines indicate treatments with Allee effects.

Table 3.10. 3-way crossed ANOVA for the effects of treatments and initial larval density (see above) on thorax lengths of male *D. simulans*.

Source	**SS**	**d.f.**	**F**	**p**
banana (B)	0.02	1	3.89	0.050
yeast (Y)	0.24	1	49.16	< 0.001
larval density (D)	0.04	3	2.97	0.032
(B) x (Y)	0.09	1	17.62	< 0.001
(B) x (D)	0.01	3	0.72	ns
(Y) x (D)	0.02	3	1.24	ns
(B) x (Y) x (D)	0.04	3	3.20	0.024
residual	1.51	312		

Table 3.11. Distribution of mould occurrence on banana across treatments; shown are the number of receptacles in all trials with mould development (total number of receptacles shown in brackets).

	active yeast	**killed yeast**
fresh banana	2 (90)	49 (90)
aged banana	6 (180)	55 (90)

Density effects on expectations for the F2 generation

The results for density effects on the expected F2 progeny showed, not surprisingly, that survivorship of the F1 generally determined the outcome (Fig. 3.7). Yet, even where an Allee effect had been non-significant in the original analysis (e.g. plain grapes) it could nevertheless make a considerable difference to the numbers of expected future progeny. On the aged banana/active yeast treatment an increase of about 200 expected offspring occurred from low to intermediate densities.

Discussion

The principal objective of this study was to determine the extent of Allee effects in a species of fruit-breeding *Drosophila*. I found Allee effects operating with both the fruit types and on both fitness parameters, survival and body size (see Table 3.12 for a general summary of results). Yet, it is clear that the occurrence and the extent of the effect depends strongly on the particular conditions of the substrate and that it is extremely difficult to resolve the relative influence of any of these. A list of the speculative conditions each fruit/treatment combination is likely to provide is given in Table 3.13.

There was a marked difference overall in survival between the two fruit types, with banana being able to support more larvae than any of the grape types. Size was unaffected by fruit type. This can be explained since in the development of *Drosophila*, larvae have to reach a critical point early in the third instar where they need to acquire enough body mass to pupate, otherwise pre-pupal mortality ensues (e.g. Gordon & Sang 1941). Once this mass is reached, larvae can pupate but there is a further time period for larvae to continue to accumulate body fat depending on food availability (see Sang 1949). While this time period can lead to varying adult body sizes, survivorship is likely to reflect more closely the nutritional quality and conditions of the growth medium than any other fitness parameter (e.g. Kearney & Shorrocks 1981). The next important factor influencing survival in my experiments was inoculation with yeast. Survivorship was lowest on the plain grapes, the only treatment without any supply of baker's yeast (*Saccharomyces cerevisiae*). It is well established that the majority of *Drosophila* species have an absolute requirement for yeast probably due to their inability to synthesise important sterols which are supplied by the yeasts (Sang 1949). It is clear, that both the presence of yeast and also the type of fruit pulp were the main

Table 3.12. Summary of results. x = not observed or not significant; + = positive density dependence; - = negative density dependence; ✓ = observed or significant; ( ) = near significant or individual observations significant in pairwise comparisons (LSD, WMW); DD = density dependence; Allee = Allee effect

Fruit	Treatment	Mould	Mean survival	Survival		Size			
				DD	Allee	DD		Allee	
						♂	♀	♂	♀
grape	plain	✓	0.19	(+)	(✓)	-	x	✓	x
grape	soaked	(✓)	0.27	+	x	+	+	(✓)	x
grape	dipped	x	0.45	x	x	x	+	x	x
banana	fresh[1]	✓	0.65	x	x	x	+	x	x
banana	aged[1]	✓	0.64	x	x	(-)	x	✓	x
banana	killed[1]	✓	0.67	x	x	x	x	x	x
banana	active[1]	x	0.61	+	✓	+	+	(✓)	x

[1] mean survival calculated from treatments in combination (see Methods)

factors influencing *Drosophila* survival before any effects of density are considered. Kearney & Shorrocks (1981), too, showed that not only the yeast but the medium upon which a yeast grows profoundly influences its value for *Drosophila* nutrition and that larval food should therefore be regarded as a yeast/medium complex. They suggest that while some yeasts can supply all nutritional requirements of larvae, others may lack essential nutrients which are then supplied by the supporting medium. Alternatively, the difference in nutritional quality of different media may be mediated by their value to the growing yeasts as has been shown for collembolans (Booth & Anderson 1979). It has been possible to rear *Drosophila* larvae on dead yeast cells alone (e.g. Delcourt & Guyénot 1911; Starmer & Aberdeen 1990), but they generally perform much better when other nutrients are supplied (Sang 1949). Although *Drosophila* nutrition has been intensively researched, there still are many gaps in the understanding of the relationship between larvae, of the different instars, yeast and the underlying medium.

Further, my results show clearly that the interaction between larvae and yeast can be more complex and may not always be as beneficial as suggested by the accepted understanding of the system. In these experiments, *D. simulans* larvae performed worse in terms of survival and size on media which contained live, active yeast cells (banana) or had been soaked in yeast-solution (grapes). Although performance was poorest in the absence of yeast (plain grapes) and better on the two above resource types, it was more enhanced still when yeast was dead (banana and possibly dipped grapes, see Table 3.13). The difficulties in interpreting results of (intra- and inter-specific) competition experiments that involve media which also support the growth of live yeast-cultures have been highlighted by Nunney (1983). Yeast development results in a whole array of factors that change over the larval feeding period: yeast presence probably increases the food resource and changes the chemical composition of the medium in ways that are difficult to quantify (Nunney 1983). I suggest that the detrimental effects observed on soaked grapes are likely to be due to an accumulation of toxic metabolites linked to fermentation. *Saccharomyces* are associated with early fermentation stages where the yeast rapidly metabolises free sugars into ethanol, either under anaerobic conditions or, in the presence of oxygen, on medium where a large excess of sugar is present (Suomalainen & Oura 1987). The sensitivity of *D. simulans* to alcohol (ethanol) is well documented: Parsons & Spence (1981), for example, demonstrated that although *D. simulans* are able to utilize ethanol as an energy source at concentrations of less than 3%, above this threshold it rapidly becomes toxic and causes both adult and larval

Table 3.13. Summary of speculative relationships between yeast, alcohol and sugar concentrations on the fruit type/treatment combinations during the duration of larval development.

Fruit + Treatment	***Saccharomyces* quantity**		**alcohol concentration**	**sugar concentration**
	active	**dead**		
plain grape[1]	none to high (variable)	none to high (variable)	low	(variable)
soaked grape[2]	low	medium	high	low
dipped grape[3]	decreasing	high	low	low on surface high in pulp
banana/active yeast[4]	increasing	low	increasing	decreasing
banana/killed yeast[5]	none	medium	none	high

[1] most variable resource type; although commercially available dessert grapes are often treated with SO_2 or fungistatic agents (Peynaud & Ribereau-Gayon 1987), some will have fungi (yeasts and moulds) adhering to surface and inside the berry (Pfaff & Starmer 1987); airborne 'contamination' with yeasts is unlikely (Rosini *et al.* 1982) but ovipositing *Drosophila* females can transfer yeasts even after long generations on 'sterile' lab medium (Begon 1982)

[2] little variation as active yeast cells in solution 'swamp' most of the effects of other fungi present; rapid fermentation of sugars diffusing out of grapes to ethanol (Pfaff & Starmer 1987); as sugars run out, yeasts are likely to die off; ethanol concentration expected to be high

[3] surplus of active yeast cells in solution rapidly metabolise sugars on grape surface, producing CO_2 (Pfaff & Starmer 1987); some fermentation occurs (pers. obs.) probably due to high sugar concentration in surface juice; as sugars on the surface run out, yeast are likely to die off, creating a visible white layer of dead cells

[4] active yeast cells are transferred with larvae from egg collecting plates; yeast will metabolise sugar and increase while their metabolic products (alcohol) are likely to increase and sugars are likely to decrease; larvae and live yeasts are likely to interact closely with each other; active yeast appears to inhibit all growth of other fungi

[5] dead yeast cells are transferred with larvae from collecting plates; this appears to be sufficient to sustain larval development although the concentration must decline with time; no *Saccharomyces* metabolism and metabolic products; some effects of other fungi

mortality. Grapes that had been soaked in baker's yeast for seven days are likely to contain high amounts of ethanol and thus represent a fairly hostile environment for developing larvae. And the lower survivorship and smaller sizes on banana inoculated with live yeast cells compared to banana with dead cells could be due to similar, ethanol-induced effects. Yet, the observation that the banana/active yeast combination is the only treatment other than soaked grapes where survival was significantly affected by density and hence competition, needs further explanation.

It is commonly believed that effects of competition in *Drosophila* will be primarily on body size rather than on survival (Bakker 1961; Grimaldi & Jaenike 1984; Jaenike & James 1991) except under conditions of extreme food shortage (Bakker 1966). My data support these findings since density-dependent effects on survival occurred only on media that also produced the smallest mean body sizes. Shortage of food could explain the response on soaked grapes, where during a week's fermentation and depletion of sugars few nutrients are likely to remain (although there should be an abundance of dead yeast). If yeasts are, as hitherto assumed, merely a food source for *Drosophila* larvae, the other significant density effect occurring with banana/active yeast, cannot be explained. Since larvae did extremely well on banana with dead cells and showed no density-dependent effects, the difference must have been caused by yeast activity. If survival indeed reflects mainly food availability then one possible explanation is that there are competitive elements in the interaction of yeast with *Drosophila*, e.g. for available free sugars or other nutrients. The significant Allee effect on survivorship in this treatment then could be explained not because larval feeding facilitates the growth of yeast (e.g. Sang 1950) but instead because it controls yeast growth . This hypothesis is further supported when the results obtained from dipped grapes are considered. Fruits in both treatments are inoculated with live yeast; banana by the transference of cultures together with larvae from collecting plates, grapes by dipping them into solution. While the quantity of yeast inoculum is large on the grapes and the yeasts get a 'head start' in developing before any larvae hatch from the deposited eggs, on the banana the inoculum is small and coincides in time entirely with the transference of first instar larvae. On dipped grapes, yeasts will immediately begin to metabolise sugars in the surface juices but then probably run out of resources and die, creating a very visible white layer of dead cells. Although first instar larvae have hatched by this time, their feeding does not appear to facilitate further yeast growth, instead they have sufficient amounts of dead yeast and an abundance of sugars and other nutrients in the volume of the half to

develop on. Food abundance may then preclude density-dependent effects (Table 3.12). In contrast, on banana with active yeast the relationship between larvae and yeast is much more interactive and may represent a 'battle for dominance'. Such an explanation has not been suggested before and the precise mechanism of yeast/larval competition requires more detailed knowledge about the different stages of nutrition of both yeast and *D. simulans* larvae. To prove the existence of such a mechanism more conclusively, further experimentation is needed.

The other mechanisms by which Allee effects in *Drosophila* have largely been explained, relate to the occurrence of moulds or other harmful micro-organisms; it has been postulated that sufficiently large numbers of larvae per surface area are required to control mould development by feeding (e.g. Allee 1938; Kearsey 1965, Atkinson 1979; Courtney *et al.* 1990). There are fungi (yeasts and moulds) that produce large numbers of mycelia which render the surface of fruits dry and fibrous and thus could potentially cause larval mortality (Kearney & Shorrocks 1981). My results for plain grapes show that in investigating the relationship between mould, survivorship and density it is extremely difficult to ascertain which factors are causative and which can be termed 'effects'. Across the first three density classes (1-16 larvae per grape half) mould decreased significantly suggesting that increasing the numbers of larvae can reduce mould development. Mould also seemed to significantly reduce survivorship. Both observations hence appear to agree with the conventional hypothesis for Allee effects, i.e. that larval co-operation is needed to control harmful moulds. A more detailed analysis however, showed that the significant effect of mould on survivorship was entirely due to the low survival and high mould cover at high larval densities. Across the densities where mould reduction coincided with larval increase no significant changes in mortality took place. This suggests that density rather than mould was the factor driving larval mortality and that mould development itself was controlled not by the initial densities (they are irrelevant if larvae die due to competitive effects) but by the actual numbers that survive. Since I do not know from the current data at what stage larval mortality occurred, the true numbers that control mould probably lie somewhere inbetween the initial density and the number of survivors. Since it was clear from the analysis that density was a causative factor, it was impossible then to determine which of the two parameters, mould or survival, was the dependent variable. As mortality was not affected over the range where mould was controlled it appears that the slight but non-significant Allee effect observed was not due to mould. This is further supported

by the significant Allee effect on survival occurring on the medium where no visible mould was present at all (banana/ active yeast). The alternative hypothesis (competition between larvae and yeast) becomes more attractive. To resolve this issue, a balanced, fully factorial design which includes an estimate of mould would be desirable. Furthermore, it may be important to control both the species of moulds present and their (relative) quantities, as the different physiologies and metabolic or chemical properties of the moulds are likely to be relevant. No attempt to quantify or identify the resident, microbial fauna could be made. The relationship between *Drosophila* larvae, yeasts, moulds (and bacteria) remains a system where many details have yet to be understood.

The body size results were by far the most difficult to interpret. Both sexes responded very differently to increases in density; females showed positive density-dependence on some treatment combinations but never an Allee effect. Males sometimes exhibited positive density-dependence and some clear Allee effects. But they also experienced negative responses which could indicate Allee effects where the optimum has not been reached or has been reached before any subsequent decrease. The varying response of the sexes is perhaps not surprising, considering that male and female *Drosophila* differ in developmental time and final, average adult size. Bonnier (1976) first observed that female *D. melanogaster* have faster development times than males but that this was mainly due to a shorter pupariation time. Bainbridge & Bownes (1981) confirmed the difference, which approximated 4 hours at 25°C, showing that in the late third instar, males take longer to form the puparium. This time difference increased over the progressive stages. The earlier eclosion of females, because of their shorter pupal period, was also tested by Bakker & Nelissen (1963) who found the same response in both laboratory and recently caught stocks of *D. melanogaster* and *D. simulans*. To explain why males and females in my experiments responded differently to conditions of the medium and to density, differences in larval not pupal development time would have to be examined. Few people have investigated such differences mainly due to difficulties in sexing early first instar larvae accurately. Nunney (1996) however, showed that in *D. melanogaster* sex differences in larval developmental time are small, strain specific and may be in either direction. In an earlier paper however, he demonstrated significant differences between the competitive abilities of male and female larvae in different strains of *D. melanogaster* (Nunney 1983). Yet, whether males or females were competitively superior was variable in different studies and hence generalisations are difficult. My data suggest that there were differences in the

two sexes competed and possibly developed but I feel that without a more detailed approach it is impossible to explain these differences.

The highly significant batch effects obtained especially on grapes emphasise that in order to detect responses, large numbers of replicates are required. For plain grapes, some of the variation is explainable, as grapes probably vary largely in their post-harvest treatment. Commercially available grapes are often but not always treated with fungicidal agents or SO_2 to prevent microbial attack and to prolong shelf life (Peynaud & Ribereau-Gyon 1987). Freezing can further kill fungal cultures (Goepfert 1980) but many moulds and yeasts are nevertheless able to persist attached to the skin or in the interior of the grape (Rosini *et al.* 1982). Ingram & Lüthi (1961) showed that it is unlikely for yeast to colonise grapes through air currents; in their estimate it would take several years before falling cells could colonise even 1 cm^2 of skin. Grapes not treated with yeast then present a very probabilistic resource, the quality of which is largely determined by the micro-fauna already present, although it is possible (but not clearly supported by my results) that ovipositing females, too, may add yeasts to oviposition sites (e.g. Vacek *et al.* 1985; Starmer & Aberdeen 1990) even when they have been in laboratory culture for many years (Begon 1982).

Finally, the results for the effects of density on the expected F2 progeny did not add many further points. I showed that while the precise control of conditions and the elimination of resource heterogeneity (banana) reduced the variation and increased the significance level of Allee effects in the system, the probably most relevant result was obtained when using plain grapes: a system where little control was exerted by the experimenter. Apart from the reduction in artificial manipulations, plain grapes are also known to be a natural resource used by *D. simulans* in the wild (e.g. Capy *et al.* 1987). And while it would be interesting to see whether the proposed mechanism of yeast/larval competition can stand up to further investigation, its likelihood to be relevant in natural population is, perhaps, more questionable.

To summarise, it is possible that through the effects on survival and size, laying eggs at intermediate densities can represent a better strategy for ovipositing females and one which could advance the spread of a gene or gene complex that promoted aggregation. In the experiments involving grapes, densities were obtained by allowing females to oviposit and adjust their clutch sizes individually, i.e. at a very low adult population

density. It is likely that the Allee effect in *Drosophila* operates only during periods of low total population sizes and that it can be detected only when large numbers of 'patches' are considered. A model that simulated a system of fruit-breeding *Drosophila* could provide the framework to conclude whether the (relatively small) Allee effect, the narrow range of conditions required for the effect to operate and the frequency of occurrence are sufficient to promote the evolution of aggregation. Importantly, the most likely and most realistic mechanism, or mechanisms, that produces an Allee effect in natural *Drosophila* populations still needs to be established and quantified.

Chapter 4 - Assessing the effects of patch quality, accessibility and light

Summary

The oviposition behaviour of seven-day old, female *D. simulans* with standardised access to mates was tested in response to light, resource accessibility, resource size and wound size; resources used were grapes. Grape size had no effect on oviposition but larger oviposition surfaces (wounds) were visited significantly more often and produced more eggs than smaller ones. Sites with restricted access were less likely to be visited, but visited sites had more eggs leading to an increase in aggregation. Light availability also affected oviposition patterns: females in darkness visited fewer sites and generated more aggregated egg distributions than when light was available. Although egg totals in dark trials were overall significantly lower than when light was available, there was no differences in the number of eggs deposited per patch in light or dark when visited patches only were compared.

Results show that flies cannot assess the absolute size of resource patches but respond to the area available for oviposition. The location of suitable oviposition sites and clutch size are probably determined by a combination of factors, including some assessment of patch quality. Accessibility of resource patches and available light are however, more important in determining the distribution of eggs.

Introduction

In the genus *Drosophila* many physical factors influence oviposition site selection, these include: surface texture (e.g. Rockwell & Grossfield 1978); colour (reviewed by Grossfield, 1978); volatile chemicals (e.g. Jaenike 1982); humidity (Spencer 1937); temperature (Fogelmann 1979) and light intensity (Wogamann & Seiger 1983). There is also some evidence that *Drosophila* respond to the presence of conspecifics. This is achieved by identifying sites previously conditioned by larval action (Chess & Ringo 1985), perception of irregularities in the surface texture as, for example, caused by the presence of eggs (e.g. Atkinson 1983; del Solar & Ruiz 1992) or of larval density (Lewontin 1955). While some of these factors, individually or in combination, are

certainly sufficient to explain how *Drosophila* could produce aggregated distributions (i.e. as proximate causes), it is not clear whether *Drosophila* respond, to colour differences, volatiles, texture or presence of conspecifics, because these convey information about the quality of patches.

If aggregating insects responded to differences in patch quality and hence to resource heterogeneity, then at least some of the widely observed aggregation of eggs would be due to oviposition preferences. In other words, if females lay more eggs on higher quality patches they might achieve higher fitness than females laying eggs without responding to patch quality. Although this explanation is attractive, it has often been emphasised that aggregation can and does occur in the apparent absence of resource heterogeneity (e.g. Atkinson & Shorrocks 1984). Atkinson (1985), for example, showed that on a grid of banana slices originating from the same fruit, field populations of *Drosophila* still generated highly aggregated distributions and that two species distributed their eggs independently over these (apparently) homogenous patches. Experiments using homogeneous patches of *Drosophila* laboratory food medium suggest similarly that aggregation is the norm in *D. melanogaster* and *D. immigrans* (Ruiz & del Solar 1986; Shorrocks 1991) and *D. pseudoobscura* (del Solar 1968). In contrast, Ives (1988; 1991) suggested that female carrion flies make oviposition decisions based on the size and quality of carcasses and aggregate eggs by laying more eggs on high quality patches. Thus if patches were equivalent, egg dispersal would be more even although possibly still aggregated.

It needs to be emphasised that aggregation is the result of two separate processes: the distribution of ovipositing females among the patches, and clutch sizes (i.e. number of eggs laid in a single visit). Atkinson & Shorrocks (1984) pointed out that if females visit patches at random and leave them with a constant probability after laying each egg, the resulting distribution of eggs will be aggregated. Experimental evidence, obtained using large numbers of flies simultaneously, indicates that female drosophilids decide to oviposit after arriving at a resource patch (discussed in Shorrocks & Bingley 1990). This implies that aggregation is entirely a product of clutch size. However, Jaenike & James (1991) showed that female visits were non-random and aggregation of larvae in a large study of mycophagous *Drosophila* resulted both from gregarious ovipositing females and production of clutch sizes greater than one. It is not clear however, how constant

leaving probabilities are for individual females or, indeed, how clutch size differences may influence aggregation.

Conspicuousness of patches is also likely to vary between resources in natural environments and this, too, has been suggested as an explanation for aggregation of ovipositing females (Sevenster 1996). Moreover, the importance of patch size has been underestimated in the past. Sevenster & Van Alphen (1996) pointed out that larger patches may be aggregations when the numbers of eggs are considered but when the densities of eggs were examined on fruits in a neotropical *Drosophila* community, bigger fruits tended to be low-density refuges. Although the degree of aggregation observed when considering densities was lower than in previous studies, it was still deemed sufficient to allow coexistence of species.

Most aggregation studies have examined egg distributions generated by large numbers of flies simultaneously and little information is available for individual behaviour and egg distributions produced by flies in isolation. This study aims to investigate the response of individuals to differences in the size of resource and in the available oviposition surface to determine how egg distributions may be influenced by light conditions and resource accessibility.

Materials & Methods

Flies

To control for some differences in fecundity I used only seven-day old female *D. simulans* in the experiment. Flies were from a wild strain collected in Zimbabwe, within the natural range of the species before it became 'domestic' (Lachaise *et al.* 1988). The stock had been established for less than two years. Females were isolated on their day of eclosion, using CO_2 as an anaesthetic, and kept together with two older male *D. simulans* on a standard *Drosophila* medium (see Appendix 1) in 25 mm x 75 mm glass vials. No further anaesthetic was used. To accustom females to grapes as potential oviposition sites, slices were added to the vials two days prior to the experiment.

Resources

The resource patches used were white, seedless grapes known to be a suitable oviposition site for *D. simulans* (e.g. Dytham *et al.* 1992). The grapes used for one experimental run always originated from the same variety and bunch, frozen on the day of purchase to equalise their state of decay and control for as much variation as possible. Before presentation to flies, intact grapes were thoroughly defrosted (2 hours in tepid water), weighed and sorted into two different size categories: small (< 2.2 g) and large (> 5.0 g). Most grapes fell between the two categories and were discarded.

Grapes were artificially 'wounded' at the top (i.e. opposite end to peduncle) to reveal the flesh of the fruit providing a suitable oviposition site. The otherwise intact skin of each grape was cut, immediately before experiments, using circular stencils of differing diameters and peeled off leaving the flesh exposed. Wound sizes were small (2 mm diameter) and large (15 mm diameter) giving four possible combinations of grape size and wound size in each trial. New grapes were prepared for each trial.

Experimental Set-up

Accessibility of oviposition sites was also investigated. Grapes were half buried, stipe end down , in moist sand, leaving the wounds exposed. The grapes were situated either at the bottom of a 25 mm x 75 mm glass vial ('in tube') or, freely accessible, in 25 mm x 11 mm plastic receptacles ('exposed'). In each trial grapes were either all 'in tube' or all 'exposed'. Trials were carried out in either totally dark ('dark') or constantly illuminated ('light') lighting conditions. A single grape of each of the four grape size-wound size combinations was randomly assigned to one of the four corners of an experimental arena consisting of a 28 cm x 17 cm x 17 cm perspex box. A single female with two accompanying males was released into the arena without anaesthesia. The arena was then left undisturbed for 24 hours in a cooled incubator at 25°C ± 0.5°C.

Egg counts and replication

After the 24 hour period the number of grapes containing any eggs and the exact number of eggs on each grape were recorded using a binocular microscope. There were 50 replicates for each treatment category ('dark+exposed', 'light+exposed' etc.) giving a total of 200 female *Drosophila* and 800 grapes.

Measuring aggregation

The spatial distribution of eggs was measured using the index of aggregation, *J*. This index is based on the idea of mean crowding following Loyd (1967) where intra-specific aggregation is measured by the proportionate increase in the number of conspecifics experienced by a random individual relative to a random, Poisson distribution (see Shorrocks & Sevenster 1995). *J* is calculated as:

$J = [(V / m) - 1)] / m$

where m is the mean number of eggs per patch and V is the variance in eggs per patch. A value of $J = 0$ indicates a random distribution of eggs, while a value of $J = 0.5$ indicates a 50% increase in the number of potential conspecifics expected on a grape half. I will use *I* rather than *J* to denote this index, because it was estimated to measure aggregation not between individuals of the same species but between siblings, i.e. to measure the spatial distribution of eggs by one female. This distinction will become more relevant in Chapter 6.

Statistical Analyses

The Scheirer-Ray-Hare (SRH) extension of the Kruskal-Wallis test (a non-parametric version of a two-way ANOVA using ranked data) was used on the number of visits because the data were clearly discrete with only five possibilities (0-4 sites visited). In this procedure the data are ranked and a standard two-way ANOVA is performed. The individual sums of squares are divided by the total of the mean sums of squares and the generated statistic is distributed as chi-square (see Sokal & Rohlf, 1995 for details).

The numbers of eggs were analysed in different ways. For accessibility and light, the number of eggs laid per female (or trial) was compared using the SRH test as these data were not normally distributed. These data included the flies which laid no eggs. To assess the effect of grape size and wound size, the proportion of the eggs laid on each grape type in every trial was calculated. These proportions were then compared using Wilcoxon-Mann-Whitney (WMW) tests. Flies laying no eggs were excluded. The values for *I* under different conditions of accessibility and light were again not normally distributed and so were compared using SRH tests.

Table 4.1. The mean number of grapes used, the mean number of eggs per female (including all trials) and the median of the index of aggregation, *I*. N = the number of females examined in each of the four treatment combinations. IQR = inter-quartile range.

Treatment	**N**	**mean visits (SE mean)**	**mean total eggs (SE mean)**	**median *I* (IQR)**
dark / in tube	50	1.02 ± 0.02	11.42 ± 0.89	2.64 (0.39)
light / in tube	50	2.15 ± 0.13	25.46 ± 2.12	0.92 (1.33)
dark / exposed	50	1.70 ± 0.12	15.44 ± 1.69	1.97 (1.82)
light / exposed	50	2.66 ± 0.46	21.82 ± 2.05	0.85 (0.89)

Table 4.2. Results of Scheirer-Ray-Hare extension of Kruskal-Wallis test on the number of grapes visited (see Sokal & Rohlf, 1995 for an explanation of the test). * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Source of Variation	**df**	**MS**	**SS/MS_{Total}**
Access	1	42369.6	13.92 ***
Light	1	170703.3	56.07 ***
Access*Light	1	1058.0	0.35 ns

Table 4.3. Results of Scheirer-Ray-Hare extension of Kruskal-Wallis test on the total number of eggs laid per trial, including trials in which no patches were located. * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Source of Variation	**df**	**MS**	**SS/MS_{Total}**
Access	1	1575.6	0.47 ns
Light	1	103922.4	31.09***
Access*Light	1	5040.1	1.51 ns

Table 4.4. Results of Scheirer-Ray-Hare extension of Kruskal-Wallis test on the index of aggregation, *I*. This parameter cannot be calculated for trials where no eggs are laid. * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Source of Variation	**df**	**MS**	**SS/MS_{Total}**
Access	1	46735.2	17.22***
Light	1	111971.7	41.26***
Access*Light	1	2246.2	0.83 ns

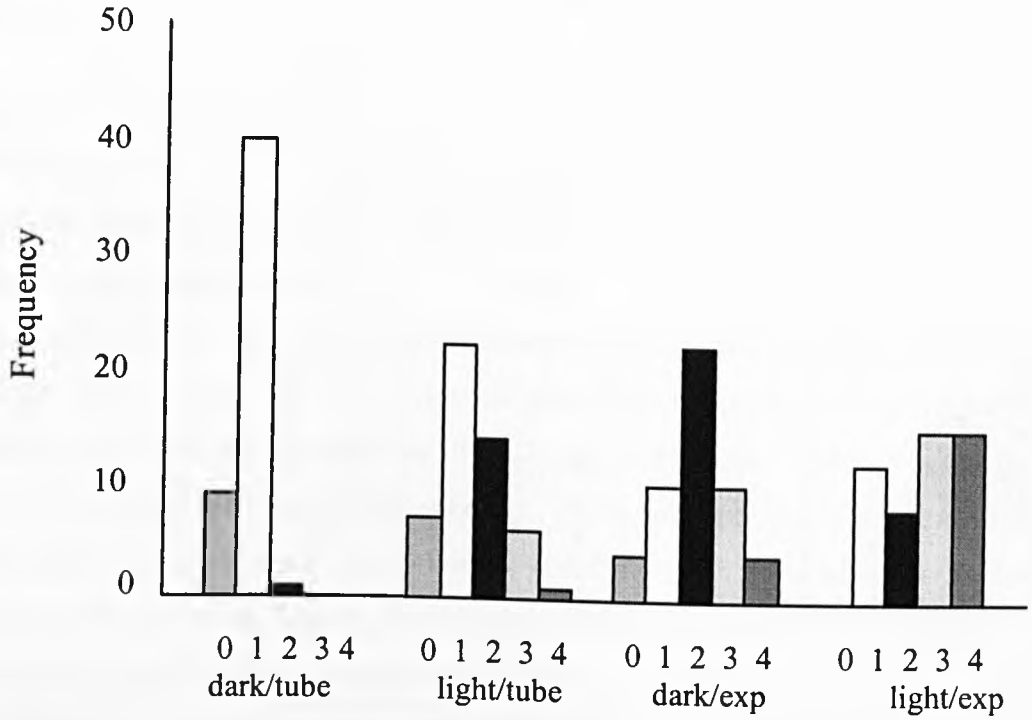

Fig. 4.1. The frequencies with which 200 female *D. simulans* used four possible oviposition sites in an observational arena over 24 hours under varying experimental conditions. tube = 'in tube', exp = 'exposed'. Differences in shading reflect the number of sites used per trial (as shown underneath the axis) to enhance ease of comparison.

Table 4.5. The mean eggs per female (± s.e), mean eggs per grape (± s.e.) and total eggs divided by two levels for each treatment (access, light, grape size, wound size). 'excl. zero trials' = trials were no eggs were deposited removed from data set; 'excl. all zeros' = all empty patches removed.

Treatment		**mean eggs per female (excl. zero trials)**	**mean eggs per grape (excl. all zeros)**	**total number of eggs**
Access	in tube	18.34 ± 1.41	11.62 ± 0.65	1639
	exposed	18.87 ± 1.38	8.52 ± 0.49	1755
Light	dark	13.48 ± 0.99	9.84 ± 0.71	1132
	light	23.56 ± 1.48	9.75 ± 0.49	2262
Grape size	large	10.25 ± 0.78	9.92 ± 0.57	1845
	small	9.61± 0.78	9.62 ± 0.56	1549
Wound size	large	13.52 ± 0.89	11.48 ± 0.56	2466
	small	5.34 ± 0.49	7.12 ± 0.45	961

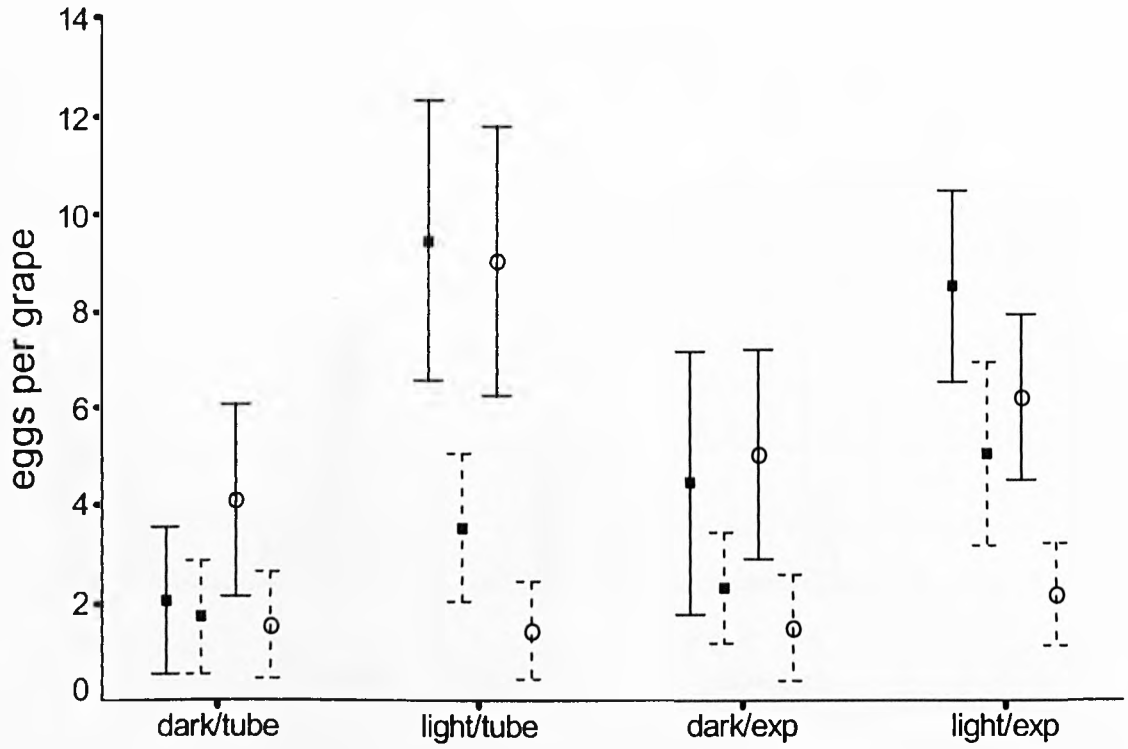

Fig. 4.2. The mean number of eggs per grape laid by single female *D. simulans* in 24 hours under varying experimental conditions. Symbols indicate grape size; filled squares = large grape, open circle = small grape, and error bars (± 95% C.I.based on s.e. mean) indicate wound size; solid line = large wound, dotted line = small wound. The data include all zero observations.

Results

Light and Accessibility

Both light and access had a highly significant effect on the number of patches visited (Tables 4.1 and 4.2 respectively). Flies in darkness oviposited on fewer patches than those in the light and even fewer sites were located when grapes were at the bottom of a glass vial. Hence the largest number of patches were used when grapes were exposed and light was available while many flies failed to located any patch at all in darkness and when sites were inaccessible (see Fig. 4.1; higher frequency of zero trials in trials without light, with restricted access or both,). The number of eggs laid by each female during a trial was also highly affected by light availability (Tables 4.1 and 4.3). Nearly twice as many eggs were laid when arenas were illuminated but access to oviposition sites had no effect (Table 4.3, column 2 in Table 4.5). However, after excluding zero observations (i.e. sites not visited), number of eggs per patch did not vary between 'light' and 'dark' whereas more eggs were laid on grapes at the bottom of vials (column 3 in Table 4.5). Egg distributions were significantly more aggregated (higher *I*) in darkness and when grape access was restricted (Tables 4.1 and 4.4).

Grape and wound size

Grape size had no effect on the oviposition behaviour of *D. simulans*. Neither large nor small grapes were preferred (Wilcoxon-Mann-Whitney text; $W = 33921.5$, $z = -1.48$, $p = 0.139$ ns). Although slightly more eggs were found on large grapes there was little difference when located patches only were considered (Table 4.5).

Wound size, had a highly significant effect on egg numbers (Wilcoxon-Mann-Whitney test; $W = 40234.0$, $z = -8.01$, $p < 0.001$) with more than twice as many eggs being laid on large wounds both overall and when considering located patches only (Table 4.5). Figure 4.2 shows that the response to grape and wound size was very consistent under different treatment conditions, although it appears that less discrimination is shown by females in darkness and with restricted access to oviposition sites.

Discussion

Light

Vision mediates many aspects of *Drosophila* behaviour (Grossfield 1978); emphasised by the rather large proportion of eye to head size. *Drosophila* are capable of detecting light intensities and use light to discern patterns. McDonald & Parsons (1973) demonstrated that activity patterns in *Drosophila* alter according to light intensity but that this relationship varies between species. In *D. simulans*, activity increases with light intensity (McDonald & Parsons 1973). As flies rely on visual cues for flight and landing (Grossfield 1978), this offers some explanation of the observation that flies visited significantly more sites and laid almost twice as many eggs in light trials. However, flies in the dark trials still managed to locate sites, if less often, indicating that senses other than vision are used. Presumably flies walked between patches in the dark and flew in the light. Interestingly, the comparison of egg numbers on located grapes only, suggests that egg laying rate is unaffected by illumination which is not in accord with Wogaman & Seiger (1983) who found that *Drosophila* strains laid significantly more eggs under either dark or light conditions, depending on the phototactic tendencies of the genetic strain.

Accessibility

Restricted access and hence also restricted ease of leaving a resource, greatly inhibited the dispersal of female *D. simulans.* This is demonstrated by the effect on number of grapes visited. However, restricted access led to higher numbers of eggs on located patches and a significant increase in the index of aggregation. The most likely explanation is that females attempting to move away from a resource patch to search for another are prevented from doing so easily and therefore continue to oviposit on the same patch. Little is known about the field ecology of drosophilids and it is difficult to evaluate under what circumstances real resource patches have restricted access. The grapes were situated at the bottom of glass vials and although thus clearly visible, a reduction in volatile chemicals arising from the sites could lead to some decline in olfactory detectability. This is strongly supported by the higher frequency of zero trials not only in darkness but also during the 'in tube' trials in contrast to trials with available light and exposed sites that all generated eggs. The importance of olfaction in locating resources is well established; many insects, including hymenopteran parasitoids (e.g. Vet & Papaj 1992), Hessian flies (Harris *et al.*1993), mosquitoes (e.g. Davis & Bowen

1994) or *Drosophila* (see Grossfield 1978; Zanen *et al.* 1994), respond to odour or odour plumes when searching for feeding and breeding sites. Thus both visual and chemical cues are important and if some sites varied in conspicuousness or olfactory detectability, (at least some) aggregation could be explained (see Sevenster 1996).

Grape and wound size

The size of the grape affected neither number of visits nor number of eggs. Although there was a small increase in egg numbers on larger grapes, once a patch was located egg numbers were almost equal between the sizes. This is surprising, since the amount of resource available to competing larvae vitally influences their viability and future fitness. Many insects, including tephritid fruit flies, seed beetles and hymenopteran parasitoids, measure host/resource size, then adjust the number of eggs laid (e.g. Mitchell 1975; Schmidt & Smith 1985; Takagi 1986; Leyva *et al.* 1991). The seed-parasitising beetle (*Stator beali*) oviposits preferentially on larger seeds when presented with a choice and reduces its clutch sizes when forced to lay on smaller seeds (Fox & Mousseau 1995). Visser (1996) demonstrated that oviposition behaviour of the gregarious hymenopteran parasitoid (*Aphaereta minuta*) is influenced by previous encounters with conspecifics. Here, flies were kept in isolation from other females and overall egg densities were fairly low. Even small grapes may present super-abundant resources for developing larvae, especially in the absence of conspecifics. While open to further investigation, it is unlikely that females evaluate egg densities in response to the total resource unit size, more likely, *D. simulans* are unable to assess the true size of a resource patch. If not influencing oviposition site choice, any effects that resource size may have on aggregation (see Sevenster & Van Alphen 1996) are unlikely to be important for the evolution of such distributions.

The results for the size of the available oviposition surface were very different: large wounds were more likely to be discovered and once discovered had far more eggs laid on them. The importance of wounds on resource surfaces is also clear in McCoy's (1962) experiments where female *Drosophila* oviposited exclusively on the moist surfaces of fresh cracks in the skin of tomatoes. Exposed surfaces allow flies to place eggs securely at oviposition, an additive genetic trait with inheritance patterns that suggest strong directional selection for a greater tendency to insert eggs rather than simply place them onto surfaces (Albornoz & Domínguez 1987). Furthermore, the exposed flesh of a fruit is colonised by micro-organisms including the yeasts on which

exposed flesh of a fruit is colonised by micro-organisms including the yeasts on which the larvae feed and cracks probably facilitate larval access to the nutrients within the fruit. Females may choose larger wounds for a variety of reasons. Any chemical cues arising from the flesh of the fruit that were used to locate the patch would presumably be more intense around a large rather than a small wound. If equal numbers of eggs were laid on large and small wounds then the egg density on the large wound would clearly be much lower. Atkinson (1983) showed that *Drosophila melanogaster,* a sibling species of *D. simulans,* are able to respond to the presence of eggs. Therefore local egg density rather than size of the resource unit may be the way in which a female fly can assess whether a patch is suitable for further oviposition. The maximum numbers of eggs laid onto large and small wounds in this experiment were actually almost equal (50 and 45 respectively) which shows clearly that lower egg numbers on small patches are not simply due to an upper limit on egg density but probably to processes discussed above.

Inferences and implications

Resource patches with no eggs can be of two types: those not found and those found but not used. This "problem with zeros" has been highlighted by Shorrocks & Bingley (1990). To distinguish experimentally between different types of unused patches is difficult, but if a very large number of potential sites is available the expected frequencies may be calculated (Shorrocks & Bingley 1990). While this is not possible with the current data, it is likely that the significant decrease in the number of patches used when accessibility is restricted or no light is available is due to an increase in the number of undiscovered patches.

These data, in accord with Jaenike and James (1991), show that the idea of random arrival at patches suggested by Atkinson and Shorrocks (1984) is unlikely if the accessibility (or conspicuousness) of patches or the available light varies. Additionally, the probability of a fly leaving a patch after each egg is not constant, as indicated by Atkinson and Shorrocks (1984), but is affected by wound size, accessibility and light. Indeed, restricted accessibility (or conspicuousness) of patches would result in increased aggregation due to a combination of larger clutch sizes and fewer visited patches whilst the size of resource units is not assessed.

Egg numbers per patch in this experiment (and in nature) are clearly the product of both pre- and post-arrival processes. They must depend on search time and conspicuousness but also on some 'judgement' of the suitability of the patch by individual flies. The significant responses to the imposed experimental conditions indicate concordance between females, but the variation that nevertheless exists between individuals suggests that other factors are also important. Further aspects of patch quality and individual behaviour will be investigated in Chapters 5,6 and 7.

Chapter 5 - Oviposition behaviour, clutch size and selection

Summary

The degree to which single female *D. simulans* aggregate their eggs over four patches (four halves of two split grapes) in 24 hours was subjected to a divergent selection experiment, high (H) and low (L) for aggregation. Aggregation was measured using the index *I*. Even after ten generations no significant difference between the selected lines was detected. Instead, significant differences were recorded between generations, indicating that oviposition patterns of individual females are highly variable and probably reflect differences in resource characteristics. A coarse-grained study of oviposition site use by isolated females showed a strong correlation between time spent on a patch and egg numbers deposited. Females preferred to be on or around oviposition sites but there were also visits to patches that did not generate any eggs. Results suggest strongly that the distribution of eggs in one experimental arena is the product of different clutch sizes, i.e. the eggs laid in a single visit.

Introduction

It is essential to establish that aggregation of eggs rather than of adults is a heritable trait with genetic variation before asking what selective mechanisms might promote such behaviour in *Drosophila* (see also General Introduction). Genetic variation among strains is the rule rather than exception with behavioural characters of *Drosophila* (Ehrman & Parsons 1974) and oviposition behaviour is no exception. Takamura & Fuyama (1980), for example, found considerable variation among different laboratory stocks of *D. melanogaster* for oviposition site choice, either on a food medium or a paper surface; a bi-directional selection experiment confirmed the genetic basis of this behaviour. Albornoz & Domínguez (1987) demonstrated that the tendency of *D. melanogaster* to insert eggs into the artificial oviposition substrate showed great genetic variation which was largely additive but also showed a strong directional dominance effect. This was expressed as heterotic effects in the direction of a greater tendency to insert eggs in hybrids than in the parental generation which suggests past directional selection pressure for insertion behaviour. Egg retention by virgin females and oviposition blocking by mated flies are also genetically determined (Boulétreau-Merle & Terrier 1986) as is substrate choice for oviposition in *D. simulans*,

D. mauritiana and *D. sechellia*; all three species showed significant intra-specific variation of mostly additive genetic character (Moreteau *et al.* 1994). Similarly, Jaenike & Grimaldi (1983) found that substrate choice for breeding sites had significant intra- and inter-specific variation in *D. tripunctata* and *D. putrida.*

The genetic basis of aggregation behaviour has been investigated. Del Solar (1968) showed that gregariousness in oviposition site choice could be selected for in captive populations of *Drosophila pseudoobscura*. Gregariousness in del Solar's experiments refers to the behaviour of adult females with respect to each other, i.e. the component of aggregation that is due to females concentrating at oviposition sites. Aggregation due to clutch size is ignored. In the design of del Solar's (1968) experiments, 15 fertilised females were allowed to oviposit for 24 hours on 15 patches of a standard laboratory culture medium. A high line (H) was established and maintained by rearing flies from 60 eggs collected from the patch(es) with the greatest number of eggs while the low line (L) originated from 60 eggs selected from patches with the lowest egg numbers. The degree of aggregation rapidly decreased in the L-line and increased in the H-line. Strikingly, fecundity increased substantially in the L-line while it remained the same in the H-line. Del Solar (1968) argues however, that fecundity is not associated with the aggregation index (measured by Charlier coefficient of disturbance). Very similar results were obtained for *D. melanogaster* by another divergent, mass selection experiment of near identical design (Ruiz & del Solar 1986). In a complete diallel mating design, Ruiz & del Solar (1993) showed that the tendency for aggregation is under polygenic control with a high proportion of additive variance and great variation between individuals and genetic strains. A dominance component directed towards an increase in aggregation (75% of hybrids showed greater aggregation than parental flies, i.e. heterosis) is suggested to demonstrate that the degree of egg aggregation is of ecological importance. Further, Ruiz-Dubreuil & Köhler (1994) showed that the genes for gregariousness are distributed over chromosomes II and III with an accumulation of factors for high aggregation (meaning gregariousness) on II and for low aggregation on III.

Aggregation therefore, has a genetic basis and can be altered by selection due to the substantial variation between females. Yet, the way in which aggregation has been selected for raises some points of interest. Del Solar (1968, *et seq.*) used artificial medium in his selection regimes and in subsequent studies examining comparative

differences between H and L lines. While food medium is useful in reducing environmental variation between experiments, artificial substrates have the drawbacks of a hard, smooth surface texture. It is known that substrate texture influences aggregation and that on smooth laboratory food, flies prefer to lay near the edges probably because they provide irregularity (Atkinson 1983). Atkinson (1983) suggests further, that females may preferentially oviposit on patches already containing eggs because eggs, too, provide irregularity. My own data imply that there are significant differences in oviposition behaviour when females face artificial or more natural substrates (see Chapter 6 for a full discussion). The very low egg numbers produced by females in the published selection studies confirm that artificial medium may not present an optimal substrate for investigations of natural oviposition behaviour. The main concern is however, that aggregation of *Drosophila* larvae can result from two separate phenomena: the aggregation of adult females among oviposition patches, i.e. gregarious behaviour of females with respect to each other, and clutch size, i.e. the number of eggs laid in a single visit (e.g. Jaenike & James 1991). The design of previous selection regimes did not allow for discrimination between the component of aggregation that is due to either factor and it is not clear whether del Solar (1968) and Ruiz & del Solar (1986) selected mainly for gregariousness or differences in clutch size. Lines were established and perpetuated using the offspring of many females simultaneously preventing the tracking of individuals (see above). Thus the contribution of offspring from one generation to the next could be either even amongst females or could be dominated by a few very fecund flies and, very importantly, could differ between the selected lines. Inevitably, fecundity must have been selected for during those experiments.

In order to produce two lines, differing in the propensity to aggregate eggs, to be used in comparative studies in this project, it was necessary to repeat but modify the design of a selection study of aggregation. I aimed to select for the degree of aggregation achieved by the distribution of eggs of individual females rather than by gregarious behaviour, a characteristic with a clear degree of variation between females (see Chapter 4). The degree of aggregation caused by an individual's egg distribution and the contribution of clutch size have been rather understudied but are likely to be very important in the generation of aggregation (see Green 1986; Ives 1988a; 1991; Sevenster 1996).

Materials & Methods

Selection

Flies used in this experiment were *D. simulans* from the wild strain collected in Zimbabwe, Africa. The stock had been maintained in the laboratory for about two years on standard *Drosophila* medium (see Appendix1). It was assumed that flies of this stock show a sufficient degree of genetic heterogeneity and are still representative of wild populations in their behaviour. The base population used to start the experiments were 100 five- to seven-day old females that had been isolated from the stock culture on their day of eclosion, using CO_2 as an anaesthetic, and kept, separately from each other but together with two older male *D. simulans* on standard medium. To accustom females to grapes as oviposition sites, slices were added two days prior to the experiments.

The selection procedure was as follows. Females were allowed to oviposit individually on four halves of two split grapes (white, seedless Spanish variety). These had been frozen on the day of purchase and thoroughly defrosted (for 2 hours in tepid water) before presenting them to the flies. Halves were half buried, cut side facing upwards, in moist sand (1.5 ml water in 12.5 g of sand) at the bottom of a 25 mm x 75 mm glass vial. An experimental 'arena' (a 17 x 11.5 x 5 cm clear plastic box) contained four vials equally spaced in one line. A single female was released into the arena (without anaesthesia) and left for 24 hours, undisturbed, in a cooled incubator at 22°C ± 0.5°C with continuous light from a source perpendicular to the line of vials thereby equalising the degree of illumination amongst the patches. To establish the two selection lines, high (H) and low (L) aggregation, females had to lay between 12 to 36 eggs in the 24 hours. This was based on data from a previous study (light/in tube, see Chapter 6) where this had represented 50% of all females. Flies not fitting the criteria were discarded. Such a constraint is necessary to avoid selecting for flies that lay either very few or very many eggs. Since the number of eggs is likely to influence their spatial distribution, females in either line could begin to differ significantly in fecundity, if not controlled.

The spatial distribution of eggs was measured using the index of aggregation, I (see previous chapter for explanation). Based on percentiles from the previous experiment, females were assigned to the H-line if they had a value of $I \geq 3.0$ (top 25%) and to the

L-line if $I \leq 1.0$ (bottom 25%). The parental generation consisted of 30 flies that had fulfilled the above criteria (15 to start each line; see Fig. 5.1). They were kept in glass vials on standard medium with twice as many males from the stock culture but removed before the next generation of females to be screened for aggregation emerged. In each generation the experimental procedure was replicated, screening as many female offspring as required until 15 females fulfilling the criteria for each line were obtained. These were again kept on standard medium in glass vials and so on.

Over the ten generations a total of 1103 females were tested; 179 were discarded because they did not lay any eggs. Although the grapes to test one generation were always from the same bunch of grapes, between generations different bunches had to be used.

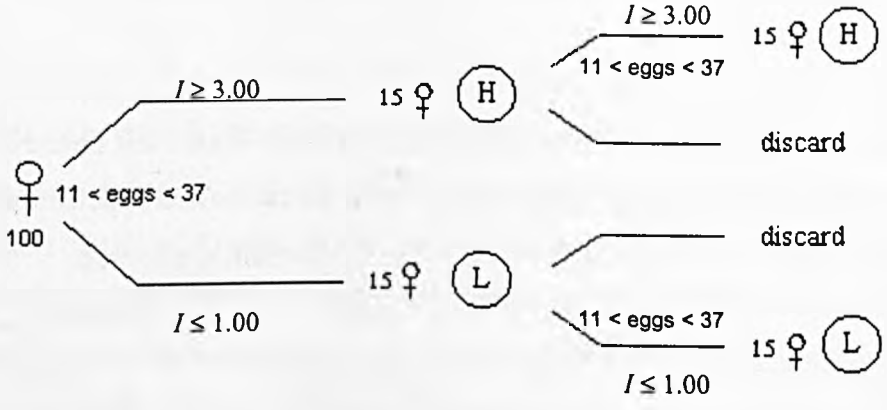

Fig. 5.1 Diagram illustrating the criteria for the two selected lines, high (H) and low (L) for aggregation.

Coarse-grained study of oviposition behaviour

Inspired by Shorrocks & Bingley's (1990) study into the simultaneous, time-dependent distribution of 30 female *D. melanogaster* over 64 grape halves, an experiment was conducted to examine the relationship between egg numbers and the time spent on

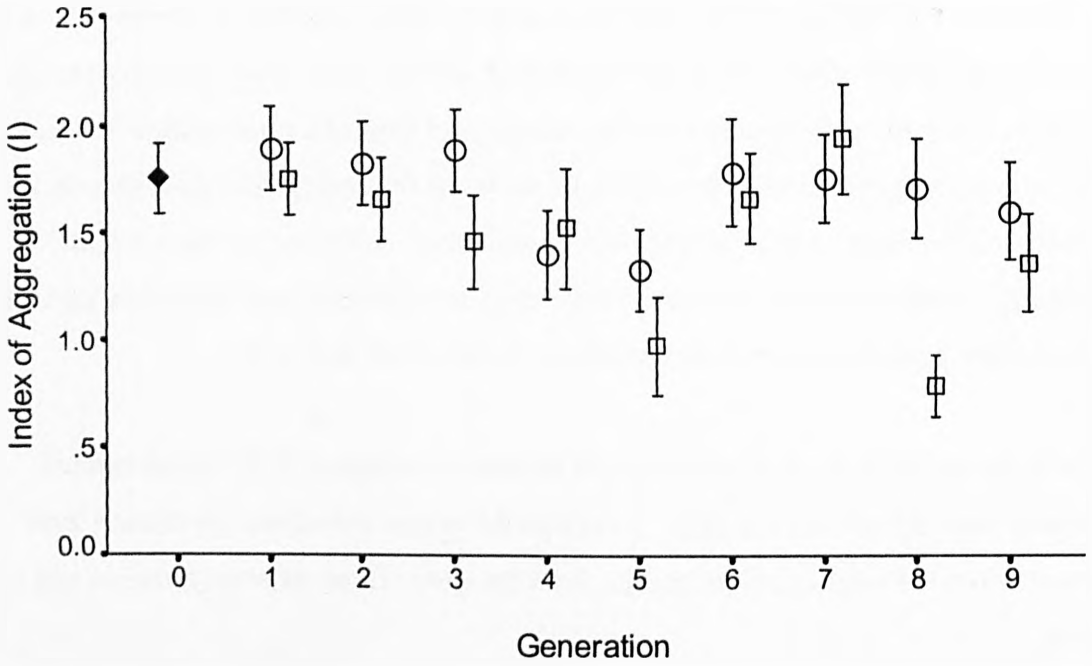

Fig. 5.2. Mean (± 1 s.e.) index of aggregation (I) for successive generations of *D. simulans* selected both for high (H) and low (L) aggregation. Solid diamond = parental; open circle = H-line; open square = L-line.

Table 5.1. Results of Scheirer-Ray-Hare extension of Kruskal-Wallis test on the index of aggregation, I, over successive generations (1-9) and for two levels of selection (high and low). * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = non-significant.

Source	**d.f.**	**MS**	**SS/MS_{Total}**
Generation (G)	8	39417.027	21.85 ***
Selection (S)	1	48580.232	3.37 ns
G x S	8	10636.545	5.89 ns

Table 5.2. Results of SHR test for the effects of generation (1-9) and selection for aggregation (high, low) on the total number of eggs laid per 24 hour trial.
* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = non-significant.

Source	**d.f.**	**MS**	**SS/MS_{Total}**
Generation (G)	8	282423.730	66.74 ***
Selection (S)	1	538117.648	15.90 ***
G x S	8	70288.514	16.61 *

patches in isolated *D. simulans* females. Using exactly the same set-up as above, 35 seven-day old females were allowed to oviposit for 8 hours on four grape halves. Every 15 min the position of a female inside the arena was recorded after her release giving a total of 32 observations per fly. Although sampling is thus clearly coarse-grained, Shorrocks & Bingley (1990) have shown that during short-term continuous observations, females moved only infrequently. Care was taken to not disturb flies while recording. Positions were 'on patch' if flies entered into any of the four tubes or 'off patch' if anywhere else in the box (sitting on the rim of a tube was recorded as off patch). As a result, it was possible to estimate the likely time spent on and off patches, the generated egg distributions and to test for an association between egg numbers and time spent around an oviposition site. Additionally, I recorded the frequency with which visits to patches were interrupted, i.e. how often females returned to patches previously visited. This allowed to establish the number of eggs per perceived visit.

Statistical analysis

As discussed in the previous chapter, neither egg totals nor the index of aggregation conform to normal distributions. The effects of generation and selection on the index of aggregation, *I*, and on total egg numbers were tested using the Scheirer-Ray-Hare extension (SRH) of Kruskal Wallis test. Associations between egg numbers and their degree of aggregation were tested using Spearman's rank correlation, as were associations between time spent on patches, egg numbers and aggregation in the second part of the study. A Mann-Whitney-U comparison was applied to the egg number per visited patch and per perceived visit.

Results

Selection

Figure 5.2 clearly shows that over all generations and for both selected lines the mean levels of aggregation were larger than zero. While eggs were on average always aggregated, individual flies sometimes oviposited near randomly or obtained negative *I* values. Before the selection started, eggs were aggregated at a mean value of $I = 1.78 \pm 0.16$ (SD). Subsequent generations differed significantly in *I* but the effect of selection on *I* was non-significant (although 3.37 is very close to the critical Chi-square value of 3.841 for $p = 0.05$ at 1 d.f. and thus only just non-significant; Table 5.1). The mean value of *I* for all generations in the H-line was slightly higher (1.54 ± 0.08) than the

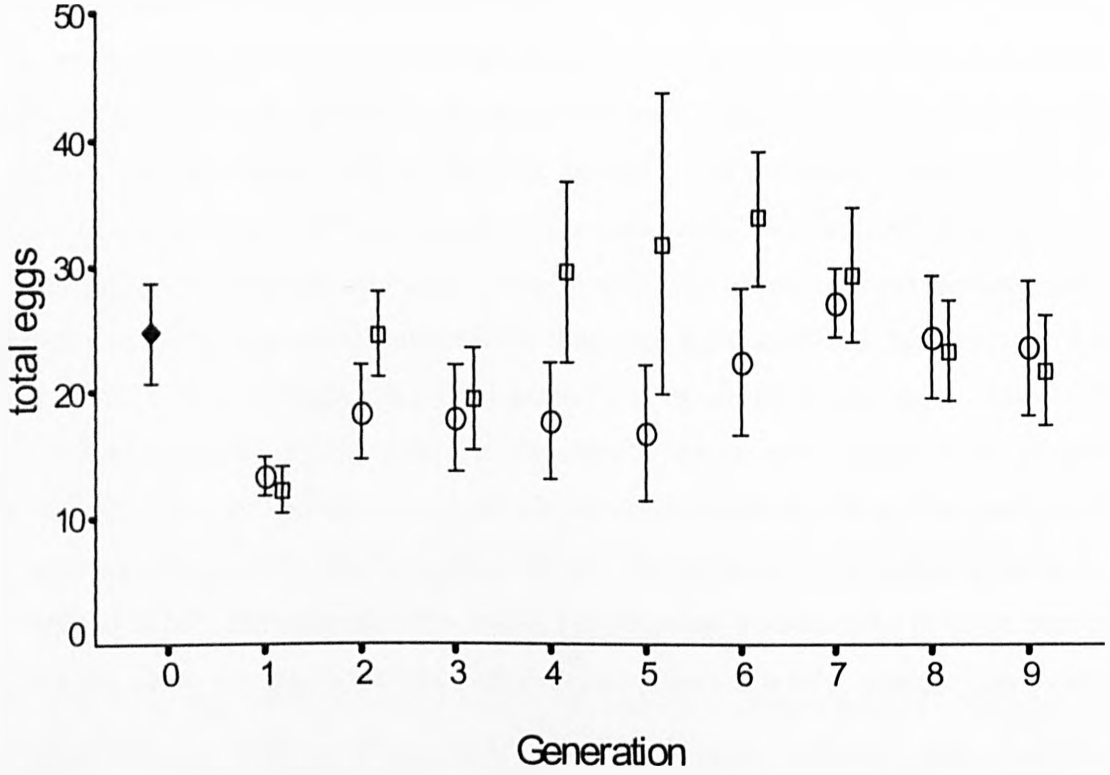

Fig. 5.3. Mean number of eggs (± 95% C.I.) laid during 24 hour trials by *D. simulans* selected for high (H) and low (L) aggregation over successive generations. Solid diamond = parental; open circle = H-line; open square = L-line.

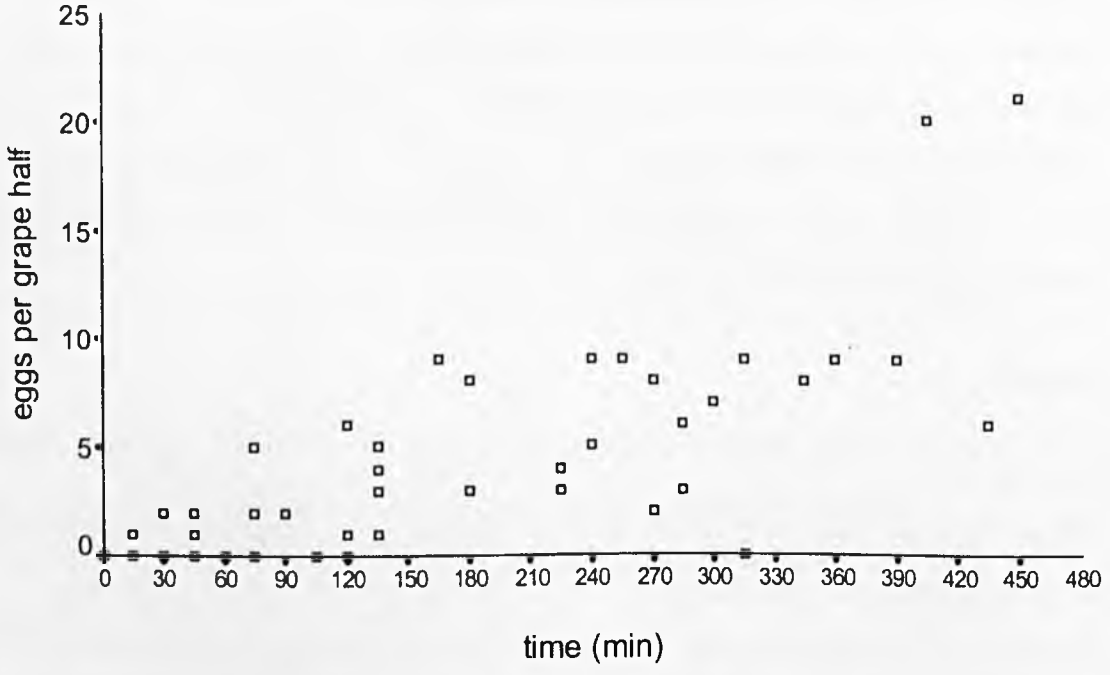

Fig. 5.4. Relationship between the time spent on four possible patches and egg numbers for 27 isolated *D. simulans* over 8 hours, sampled every 15 minutes (Spearman's rank correlation; $r_S = 0.70$, $p < 0.001$, $N = 55$).

parental average as was the mean for the L-line (1.30 ± 0.08). Hence although there was some near significant response to selection with slightly lower values of *I* in the L-line than in the H-line, the main differences were between generations. The number of eggs deposited in one trial varied greatly and was highly affected by both generation and selection for aggregation (Fig. 5.3, Table 5.2); the significant interaction term indicates that the response was not consistent, i.e. whether a female was in the H or L-line had different effects on egg numbers in different generations. Females in the L-line deposited on average (but not always) more eggs than those in the H-line (mean for all generations 23.41 ± 13.53 (SD) in the L-line compared to 19.92 ± 12.75 (SD) in H) but the largest amount of variation again was seen between generations.

The differences in fecundity displayed by females did not affect the spatial distribution of eggs in any consistent way as can be seen when comparing Figs. 5.2 and 5.3. Spearman's rank correlation confirmed that there was no significant association between the total number of eggs laid in a trial and the generated index of aggregation (r_S = - 0.248, p = 0.62). The relationship is complex however. *I* is clearly influenced by both the number of eggs laid in total and the number of patches used. Thus for flies that oviposited on either 1, 2, 3 or 4 patches there is an obvious positive correlation between total number of eggs and aggregation within each group, e.g. the more eggs are laid on one patch only, the higher *I*, the more are laid on two patches, the higher *I*, and so on. In addition, as the mean number of eggs laid increased more grape halves were used by individual females (Table 5.3), yet due to great variation in individual behaviour this pattern generated no significant association between egg total and *I*. Thus differences in fecundity lead to some changes in aggregation but overall those differences show no consistent pattern.

Oviposition behaviour

Of the 35 examined females, 8 (22.86%) failed to produce any eggs during the 8 hour-trials although 2 of these did spend some time in the glass tubes. Most flies moved onto a patch within 2 hours. Only in once case did a patch that had not been recorded as visited contain eggs. Descriptive statistics for egg numbers per patch, per perceived visit and per female as well as the time spent on patches are given in Table 5.4. Females rarely broke up their visit to a patch demonstrated by the non-significant difference in egg numbers per patch and egg numbers per perceived visit (MWU; Z = -0.759; p = ns,

Table 5.3. Frequency of the number of grape halves containing eggs per trial and the total number of eggs as well as mean eggs oviposited (SD) by isolated females in the high (H) and low (L) selection line.

	H-line			L-line		
grape halves used	**frequency**	**eggs**	**eggs per female (SD)**	**frequency**	**eggs**	**eggs per female (SD)**
1	130	1651	12.7 (11.1)	95	1587	16.7 (12.9)
2	151	2763	18.3 (12.3)	140	2870	20.5 (12.4)
3	115	2749	23.9 (13.4)	105	2793	26.6 (13.9)
4	90	2674	29.7 (12.9)	98	3047	31.1 (13.0)
Total	486	9837		438	10297	

Table 5.4. Descriptive statistics for egg numbers, time spent on patches and aggregation of 27 individual *D. simulans* observed every 15 minutes for 8 hours. N = number of observations in sample.

Variable	**N**	**mean**	**± SE mean**	**range**
visits	27	2.04	0.15	1 - 4
eggs / patch	55	3.92	0.61	0 - 21
eggs / perceived visit	55	3.40	0.60	0 - 21
eggs / female	27	8.00	1.01	1 - 21
time / patch (min.)	108	82.92	11.37	0 - 450
total time on patches (min.)	27	331.67	21.13	30 - 480
% of total time on patches	27	69.10	4.40	6.25 - 100
aggregation (*I*) of eggs	27	2.29 (median = 2.00)	0.22	0 - 3.81

N = 110, see Table 5.4). Flies clearly preferred to be on a patch or at least within the tube with 69.10% of the total time spent on average on patches (Table 5.4). Egg numbers were significantly correlated with time spent on a patch (Spearman's Rank Correlation; $r_S = 0.85$, $p < 0.001$, N = 108) for eggs per patch and time spent per patch; note that observations are not fully independent as each female contributes four observations), and overall the longer females spent on patches the more eggs they produced in total (Spearman's Rank Correlation; $r_S = 0.70$, $p < 0.001$, N = 27; see Fig. 5.4). The aggregation index, *I*, was not associated with any parameter (eggs per female, time spent on patches or percentage of total time spent on a patch). Flies that were 'on patch' did not always sit on the surface of the grape but were frequently seen either in the moist sand around the fruit or close-by on the glass.

Discussion

The study, although not producing two distinctly separate aggregation lines, generated some clear and interesting results. In the absence of conspecifics and hence competition females exhibit marked differences in the way they disperse their eggs over available oviposition sites. The near significant response to selection indicates that this dispersal is probably under some genetic control. Initially the aggregation index, *I*, slightly increased in the H-line and decreased in the L-line. However, the response is not rapid as expected when selecting for traits that have a normal degree of genetic variability within a population nor is it consistent or very marked. The effort to sample 15 flies fulfilling the selection criteria for each line remained constant in each generation. Although some traits in *Drosophila* do not immediately respond to selection (e.g. Maynard Smith & Sondhi 1960; Toro & Charlesworth 1982) these are exceptional. The most likely explanation for the lack of response in these experiments is that there was a large degree of non-genetic variability that influenced egg distributions which is clearly supported by the highly significant and overriding effect of generation on the parameter, *I*.

In the previous chapter, I have shown that the propensity of female *D. simulans* to leave a patch once they have located it could be altered by several factors and is likely to be non-random. In the current scenario, it is not possible to distinguish precisely whether the numbers of eggs deposited in one patch are the product of a single visit, i.e. a clutch, or present the product of oviposition during several visits. The results of the

second part of the study demonstrate that the number of eggs however, are closely associated with the time spent on a patch and that visits, during the eight hours of the study at least, were seldomly disrupted. This suggests that distributions of eggs are the products of different clutch sizes, where a clutch is defined as the number of eggs laid in a single visit. There is no obvious reason to indicate that a different result would be obtained over the 24 hour period of the selection trials. However, if patches became saturated with eggs over a longer time-period or if (intra- or inter-specific) competitors were present, a different outcome could be obtained.

The notion that clutch size is influenced largely by environmental variables and only weakly by genetic factors is not new. Fox & Mousseau (1995) showed that in the seed beetle (*Stator beali*) clutch size, although variable and showing some weak heritability, was largely influenced by environmental factors. In fact, clutch size is generally regarded to be highly variable and dependent on many factors which has lead to some authors considering clutch size in insects in the context of optimal foraging strategies (e.g. Skinner 1985; Mangel 1987). Female size or current egg load, distribution of patches, quality of oviposition sites, density of ovipositing females but also larval behaviour and competition affect clutch sizes and the evolution of clutches is closely linked to other life-history characteristics (Skinner 1985; Parker & Begon 1986; Godfray 1987; Mangel 1987; Ives 1989).

The results of this experiment suggest that variation between generations was the most significant factor influencing both the total number of eggs deposited per female and their distribution. Despite no decrease in the amount of variance within selected lines, females within generations responded largely 'unanimously'. This suggests that the properties of the resource itself rather than differences between females (e.g. egg load, size) were the source of uncontrolled variation. Grapes within one generation were all from the same bunch but bunches varied between generations. Qualitative differences between the grapes of different bunches but also within bunches are likely to have strongly influenced the oviposition behaviour of female *D. simulans* in these experiments. This is supported by the substantial proportion of grapes (23.2%) in the second part of the study which were visited but did not receive eggs. Shorrocks & Bingley (1990) obtained a very similar proportion (8-25%) of empty patches in their study of oviposition behaviour in groups of 30 female *D. melanogaster* on grape halves. They estimated that of the empty patches, up to 58% were probably unsuitable despite

the 'similar look' of grapes in the experiments. I have shown in Chapter 3 that there are substantial differences in the quality of non-yeasted (plain) grapes as indicated by large variation in survivorship of larvae hatched from grape halves some of which originated from the selection experiment. Some causes of differences in commercially available grapes were suggested. Yet, further investigation is required to decide how closely the oviposition choice of females actually reflects the survival chances of their offspring and on what factors qualitative differences in grapes are based.

Finally, the way in which the aggregation generated by individual flies may be linked to aggregation produced by gregariousness of females must be explained. Del Solar & Ruiz (1992) demonstrated that, on artificial substrates, females that tended to concentrate their oviposition individually also had lower aggregation indices when the combined oviposition of several such females was considered. Likewise, females that dispersed eggs widely as individuals had more dispersed distributions as a group. This was demonstrated using flies not from the H and L selected lines but by testing the original laboratory stock culture which prevents any interpretation of the results in a genetic context. It is difficult to imagine how the two processes are linked as they involve very different behavioural responses. In the following chapter, I will consider this link more closely.

Chapter 6 - Clutch size, gregariousness and aggregation: investigating the links

Summary

Oviposition behaviour of *D. simulans* was investigated in response to a number of factors including male presence or absence, pre-experimental adult density (high and low) and oviposition substrate (plain, yeast-soaked, yeast-dipped grapes, artificial culture medium). Female responses were tested individually, in pairs and in groups of 20. Egg numbers and spatial distribution patterns were recorded on empty patches, patches previously used by conspecifics or by a hetero-specific competitor (*D. melanogaster*) in four-day trials. Male presence and pre-experimental adult density had little effect but substrate type significantly altered female oviposition behaviour. Egg numbers and their aggregation were higher on good-quality substrates than on those of poorer quality. Females avoided ovipositing on sites already containing eggs on all but artificial substrate. Despite avoiding each other, egg distributions were still aggregated. Decreasing the number of high quality patches increased patch use overlap. Females did not discriminate between eggs of conspecifics or their own. They did respond to eggs of a hetero-specific competitor in so far that previously used sites were less often avoided than recorded for conspecifics. Instead egg distributions of the two species were randomly associated. Results are discussed relative to previous studies of oviposition behaviour and aggregation.

Introduction

In the previous chapter, I demonstrated that individual females can produce egg distributions that are aggregated to varying degrees by generating different clutch sizes. The extent to which eggs are aggregated appears to be highly variable, is under little genetic control and is susceptible to variation in the quality of the available breeding sites (see Chapters 4 and 5). It has, however, been possible to select for aggregation in laboratory experiments but research has focused on the gregarious behaviour of females in groups, i.e. the degree to which they are attracted to the breeding sites already used by conspecifics or to each other (e.g. del Solar 1968; Ruiz & del Solar 1986). Aggregation is however, the product of two separate mechanisms: clutch sizes (aggregation caused by individuals) and gregarious behaviour, i.e. attraction among

females (Green 1986; Ives 1988a, 1991; Jaenike & James 1991). Del Solar & Ruiz (1992) demonstrated that females which tended to concentrate eggs onto one or a few oviposition sites, also generated more aggregated distributions as a group. This implies that the two processes are linked but the issue can only be resolved if the behaviour of individuals is tracked. There are weaknesses in aggregation theory because the role of the individual has been ignored; Shorrocks & Rosewell (1986, 1987), for example state that to understand guild size relationships in *Drosophila*, which are based on the aggregation model of coexistence, detailed knowledge of the behaviour of individual females is essential (see also Shorrocks & Bingley 1990). To understand the evolution of aggregation, it is imperative to study the behaviour of individuals. This study aims to explore the link between behaviour of individuals in isolation and group situations.

Oviposition behaviour cannot be studied in total isolation since *Drosophila* obviously need suitable oviposition substrates. The previous two chapters demonstrated that oviposition patterns are highly variable and are influenced by many factors. The breeding site specificity of some *Drosophila* species is known (e.g. Atkinson & Shorrocks 1977; Starmer *et al.* 1981) as are aspects of host choice or oviposition preferences of different species (reviewed, e.g. by Courtney *et al.* 1990). Yet, it is little understood how characteristics of breeding sites may influence the oviposition patterns generated by individuals. Studies that have looked at individual behaviour typically use artificial substrates, which for a variety of reasons differ from natural resources (del Solar & Ruiz 1992; Ruiz-Dubreuil *et al.* 1994). The influence of different substrate types on oviposition behaviour are explored in this study. Other factors that may be important in oviposition and aggregation are also investigated. The possible role of aggregation pheromones in oviposition, produced by male *Drosophila* but also transferred to females during mating, has been suggested in several studies (Spieth 1974; Bartelt *et al.* 1986; Schaner *et al.* 1987). Jaenike *et al.* (1992) demonstrated that volatile compounds, similar to those that can be extracted from a variety of *Drosophila* species, applied to mushrooms in the field can increase the number of species captured. They suggest that such compounds may influence the distribution of flies across breeding sites in the field; attraction to pheromones has also been demonstrated in windtunnels in the laboratory (e.g. Hedlund *et al.* 1996). In some insects, the adult population density experienced prior to oviposition site choice affects behaviour, e.g. in parasitoid wasps (Visser 1996; Visser & Rosenheim 1998). It is likely however, that adult female density (and egg density) is important at, rather than before, oviposition

and this has important implications for aggregation theory and the stability of species coexistence (discussed in Sevenster 1996).

Materials & Methods

Flies and oviposition substrates

The flies used in these experiments were *D. simulans* collected in Zimbabwe, Africa. The stock had been maintained in the laboratory for about three years on standard *Drosophila* medium (see Appendix 1). Females were isolated from the stock culture on their day of eclosion, using CO_2 as an anaesthetic, and kept together with two males on standard medium. Replicating the set-up of the selection experiment (previous chapter), five- to seven-day old females were released individually, without further anaesthetic and without the males, into experimental arenas containing four food patches. Food patches were either grapes or 'plugs' of standard medium. Grapes (white, seedless Spanish variety) had been frozen on the day of purchase. They were defrosted thoroughly for two hours prior to trials in tepid water and then split into two halves so that the four grapes within one arena originated from two split grapes. Halves were used either untreated ('plain'), soaked for seven days in 1% solution of baker's yeast (*Saccharomyces cerevisae*) ('soaked') or dipped briefly prior to a trial into 1% yeast solution ('dipped'). A further treatment consisted of giving females a choice between two patch types in one experimental run: two grape halves were 'plain' while the other two were 'dipped' (but excess moisture was dried off to give a more similar surface to 'plain') thus introducing resource heterogeneity. Grape halves were buried in moist sand (1.5 ml water in 12.5 g of sand), cut side exposed, at the bottom of a 25 mm x 75 mm glass vial. Food plugs consisted of 1.5 ml of standard *Drosophila* medium (see Appendix 1) poured into small, circular plastic receptacles (25 mm diameter x 11 mm deep) that were also placed on moist sand at the bottom of glass vials ('artificial'). An experimental arena (a 17 x 11.5 x 5 cm clear perspex box) contained four vials equally spaced in one line. Females were allowed to oviposit for 24 hours in a cooled incubator at 22°C ± 0.5°C with continuous light from a source perpendicular to the line of vials.

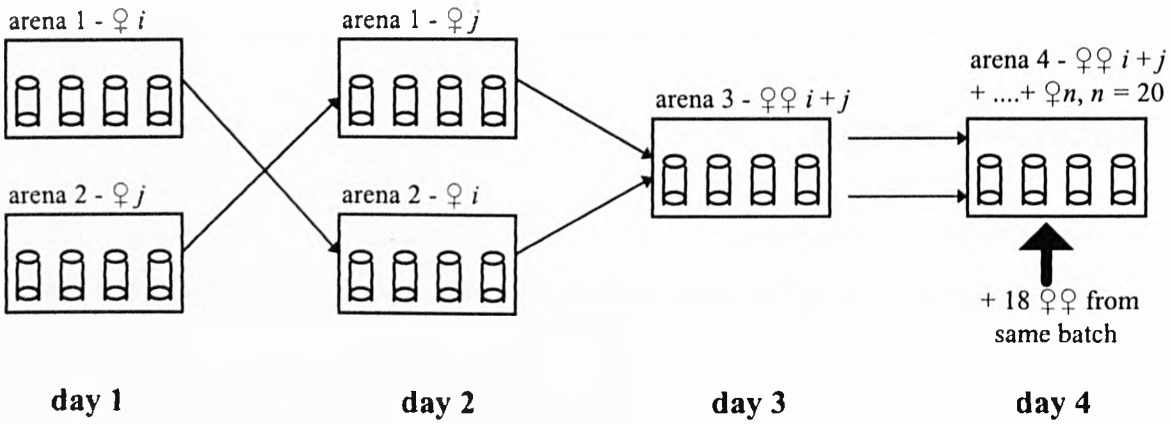

Fig. 6.1. Diagram illustrating the basic experimental procedure over the four days.

Table 6.1. Summary of basic experimental procedure for one experimental run and associated parameters that were measured. See text for explanation. Each batch involved 30 arenas being set up for day 1 and 2, approx. 15 for day 3 and 1 arena for day 4.

day	set-up	parameters	
		totals	**indices**
1	♀ *i* - arena 1 ♀ *j* - arena 2	• $Total_1$ for ♀♀ *i* and *j* respectively • $Total_{independent}$ for ♀♀ *i* + *j* super-imposed (arena 1+2) • $Total_{20}$ for super-imposing eggs of 20 random ♀♀ in batch	• I_1 for ♀♀ *i* and *j* respectively • $J_{independent}$ for super-imposing egg distributions of ♀♀ *i* + *j* (arena 1 + 2) • J_{20} for super-imposing eggs of 20 random ♀♀
2	♀ *i* - arena 2 (eggs of ♀ *j* present) ♀ *j* - arena 1 (eggs of ♀ *i* present)	• $Total_2$ for ♀♀ *i* and *j* • $Total_{sum}$ for eggs of both ♀♀ added	• I_2 for ♀♀ *i* and *j* from added eggs • J_{sum} for arena 1 and 2 (all eggs) • C_{ij} for arena 1 and 2 for association of offspring ♀♀ *i* + *j*
3	♀♀ *i* + *j* together simultaneously on fresh resource patches	• $Total_{together}$ for eggs of ♀♀ *i* + *j* in one arena	• $J_{together}$ for ♀♀ *i* + *j* in one arena
4	20 random♀♀ of one batch in a single arena on fresh resources	• $Total_{all}$ for 20 ♀♀ in one arena	• J_{all} for 20 ♀♀ in one arena

Basic set-up

One experimental trial lasted four days (see Fig. 6.1). 24 hours after introduction into arenas (day 1), females were pooted out and kept separately in clean glass vials containing only a moist paper towel. The egg numbers on patches were counted and then returned to arenas in exactly the same order as previously but females were swapped over so that female 1 was now left to oviposit for a further 24 hours on patches previously used by female 2 and vice versa.

On day 2, females were again removed and the new egg numbers on each patch were counted. The distribution of added eggs (estimated as I_2) as well as the total number of eggs across patches and their dispersal, J were calculated. *J sensu stricto* is estimated exactly as I but using the egg distribution of two conspecific females (i and j) rather than of individuals. To establish the overlap in patch use for female i and j, the index of aggregation, C_{ij} was calculated. The parameter C, as used by Ives (1988, 1991), measures the degree of inter-specific aggregation by estimating the proportionate increase in the number of hetero-specific competitors relative to a random association. It is measured as:

$C_{ij} = Cov_{ij} / m_i m_j$

where Cov is the covariance of eggs on patches between a pair of species (i and j), the subscripts indicate the species and m_i and m_j are the mean numbers of eggs per patch of species i and j respectively. When $C = 0$ the two species are randomly associated while $C > 0$ indicates positive and $C < 0$ negative associations. If C is applied to the egg distributions generated by two conspecifics, the parameter can equally measure the degree of association between individuals.

On day 3, females i and j were allowed to oviposit together, i.e. two females were released into one experimental arena containing fresh resource patches. After a further 24 hours the number of eggs per patch were counted and the distribution determined for both flies as $J_{together}$. The numbers ($Total_{together}$) and distribution of eggs generated by the two flies simultaneously can be compared to that generated artificially from egg numbers on patches produced by the same flies independently, i.e. on day 1 ($Total_{independent}$, $J_{independent}$).

The experiment was carried out in batches where 30 arenas were set up at one time and the final parameters measured (day 4) were the egg numbers ($Total_{all}$) and distribution

(J_{all}) generated simultaneously by 20 random females of one batch on fresh resource patches. Table 6.1 summarises the experimental procedure and parameters measured. Grapes for one batch were always from the same bunch of grapes but different bunches of the same variety had to be used for different batches.

In addition to varying resource types, several further factors were investigated using the standard protocol with slight modifications.

Males

The effect of male presence on oviposition patterns was tested in two ways: 1) The two males with which each female had been kept for the 5-7 days since her eclosion were released into the arena together with the female and males were kept in arenas for the first two days of the procedure; 2) Prior to each run, two male *D. simulans* each were released into two of the four resource patches. Vials were stoppered with cotton wool and kept in the incubator for 24 hours. The other two vials were kept under the same conditions but without male flies present.

Pre-experimental density

Egg numbers and distributions produced by females in the basic set-up were contrasted with those generated by females which had been kept at high adult density since their day of eclosion. All 30 females of one batch were kept together with 60 male flies in a standard, 25 mm x 75 mm glass vial on culture medium but then released individually into an arena. Flies were slowed down to facilitate manipulation by placing the tube briefly (5 min.) into a refrigerator at 4°C.

Using the same female and consistency of oviposition patterns

Instead of releasing female *i* into the arena previously used by female *j*, female *i* was re-exposed to her own eggs on day 2. The measured parameters therefore were I_1, I_2 and I_{sum} for one individual female but to avoid confusion I_{sum} was referred to as J_{sum}. Similarly, C_{ii} was referred to as C_{ij}.

To test for consistency in oviposition patterns, females were allowed to oviposit for 24 hours on four resource patches (plain grape halves only) as in the basic set-up. They were then isolated and released into a new arena containing fresh grape halves. Consistency in egg numbers and distributions from day 1 and 2 were compared.

Table 6.2. Summary of all factors included in the analysis, the corresponding batch numbers and the total number of females examined (N_{total} = 684) for each level of treatment.

factor	**level**	**batch numbers**	♀♀ **in sample**
medium	plain	1, 2, 3, 12, 14, 17	159
	soaked	4, 25	56
	dipped	5, 6, 8, 9, 13, 15, 16, 18, 19, 20	285
	artificial	7, 10, 11	64
	mixed (plain/dipped)	21, 22, 23, 24	120
males	absent	all except below	639
	present	3	25
	conditioned	8	28
density	low	1, 2, 3, 4, 5a, 6, 18 - 24	366
	high	5b, 8 - 17	318
species	*D. simulans* only	all except below	564
	mixed	9, 18, 19, 20	120
same ♀	no	all except below	624
	yes	26, 27	60

Two species

Finally, the behaviour of *D. simulans* females was tested, using the basic set-up, where the second female (female *j*) was not conspecific but instead *D. melanogaster*. *D. melanogaster* is a sibling species of *D. simulans* and the stock culture originated from females caught in Cameroon, Africa that had been maintained in the laboratory on standard culture medium for less than one year. Designation of parameters is also, strictly speaking, different, although formulae still apply: I_1 and I_2 (and corresponding totals) remain the same but instead of measuring J_{sum}, only C_{ij} *sensu stricto* is estimated. For comparative reasons however, J_{sum} is still used and presented although it represents the total aggregation of eggs by two separate species.

Levels of replication

Since the experiment involved a large number of factors it was not possible to make the design fully factorial. Different subsets of data had to be compared to investigate the effect of different factors. Table 6.2 shows the level of replication and number of batches that included various combinations of all factors: resource type, presence or absence of males, high or low female ageing density, species assembly and use of the same female on consecutive days. A total of 783 females were examined in 27 batches of which 99 laid no eggs and were thus removed from the data set. Batches using artificial medium as resources included more than 30 females as there was a higher proportion of females that laid no eggs. Experiments were continued until there were 30 females in each batch.

Consistency in oviposition behaviour of females on two consecutive days was investigated separately; a total of 60 females was examined.

Statistical analysis

Data were analysed in a variety of ways. Parameters generally deviated significantly from normal distributions. The indices of aggregation, I, J and C_{ij} have distributions with an upper limit determined by the number of patches in the system while the lower limit depends on the number of eggs laid in total. Both patches and maximum egg numbers are clearly limited by the protocol and indices were never normally distributed. Egg totals generated distributions with positive skew (g_1), were obviously discrete and were analysed, using non-parametric statistics.

The effects of males, density and using the same female twice on all parameters (see Table 6.1) were investigated first. Male conditioning of patches was tested using the Chi^2 test for association between patches that had contained males or not and the presence or absence of eggs. The other way to test the effect of male presence was to compare trials where two males were present to those where they were absent using Mann-Whitney-U test (MWU). The effect of keeping females at high or low density prior to experiments was also investigated by MWU tests as were the effects of using the same female or different females on re-introduction into arenas.

In the consistency experiment, female behaviour was tested using the Wilcoxon-signed rank tests for matched pairs (WSR). Spearman's rank correlations were used to test for associations between parameters of one female, e.g. $Total_1$ with I_1 or $Total_2$ with I_2.

The effect of resource type on parameters was tested in several ways. Firstly, the oviposition behaviour of individual females over the first two days of the experiment was analysed, using WSR test on I_1 and I_2 as well as on $Total_1$ and $Total_2$ for each food type. This enables assessment of whether female behaviour changes in a pattern that is consistent. It should be emphasised however, that individuals are not exposed to equal (controlled) conditions on day 2 but instead are exposed to different egg numbers and distributions. Additionally, parameters I_1 and I_2, $Total_1$ and $Total_2$, were checked for associations (using Spearman's rank correlation) to investigate whether females show consistency in egg output and spatial distribution regardless of changing conditions. Secondly, the change in egg numbers and distributions in arenas was analysed, using a Kruskal Wallis test (KW) for the effect of medium on all parameters. MWU tests were applied to find significant differences between pairs of different substrates. To investigate preferences of females for patch type in the mixed resource scenario WSR tests were applied to egg numbers on plain and dipped during trials. Associations between parameters observed in arenas were tested using Spearman's rank correlations carried out separately for different resource types, to reveal how aggregation patterns generated by more than one female are linked to the behaviour of individuals.

The generation of variables by artificially combining egg numbers of two females or of 20 females was compared to those generated by real assemblies (e.g. $Total_{independent}$ with $Total_{together}$, J_{20} with J_{all}, etc.), using WSR tests since variables of either type were not independent.

Table 6.3. Chi^2 arrangement and results to test for the effect of male conditioning of patches on whether or not patches were chosen for oviposition (resource type = dipped grapes). Eggs = presence of eggs on patches, no eggs = no eggs present.

	eggs	**no eggs**
males	34	22
no males	30	26

$\Sigma\chi^2$ with continuity correction = 0.328

d.f. = 1

p = 0.57 ns

Table 6.4. The results of MWU comparisons for presence or absence of males during trials on total eggs per female and indices of aggregation (resource type = plain grapes). N = females in sample.

variable	**N + ♂**	**N - ♂**	**Z**	**p**
I_1	25	45	-1.46	ns
I_2	25	44	-1.53	ns
J_{sum}	25	45	-0.82	ns
C_{ii}	25	44	-0.95	ns
$Total_1$	25	45	-2.35	0.02
$Total_2$	25	45	-0.72	ns

Finally, the effect of mixing species (*D. simulans* and *D. melanogaster*) on parameters was tested by KW test (and subsequent pairwise MWU tests) for trials that consisted of *D. simulans* only to those where the first females was either *D. simulans* or *D. melanogaster*. In the mixed scenario, response of individuals to the presence of eggs of a non-conspecific competitor was tested, using WSR tests. Associations between parameters in arenas were investigated by Spearman's rank correlations.

Results

Although the main objective of the investigation was to test the effects of substrate type and the association between parameters, other factors were tested and are presented first. By identifying non-significant effects, subsets of data for the main analysis could then be amalgamated to gain more degrees of freedom.

Males

Conditioning of patches by the presence of males prior to trials had no effect on whether or not they were chosen by females for oviposition (Table 6.3). When egg numbers and dispersal were compared for trials with or without the presence of two males, eggs per female on day 1 ($Total_1$) were significantly higher (MWU test, see Table 6.4) for trials without males (mean = 20.35, SD = 8.98 compared to 14.86, SD = 9.26) while all other parameters, including all measures of egg dispersal were unaffected (Table 6.4).

Pre-experimental density

Whether females were aged at high densities or in isolation had no significant effect on any of the parameters, although the MWU comparison for the index of aggregation on day 1 (I_1) on dipped grapes was very close to the 5% significance level with females coming from high density conditions aggregating their eggs slightly more than those coming from isolation (MWU test; Z = - 1.958, p = 0.068, N = 83).

Using the same female and consistency

The egg numbers and distributions generated by females that were re-exposed to their own eggs on day 2 were indistinguishable from those generated where females were swapped. This was true for comparisons on both plain and dipped grapes.

Table 6.5. The effect of oviposition substrate (0-4) on the total number of eggs per arena (except $Total_2$ = eggs added by ♀*j*) investigated by KW test. 0 = plain grapes; 1 = soaked grapes; 2 = dipped grapes; 3 = artificial medium plugs; 4 = mixed grapes (plain, dipped). ≈ = non-significant in MWU comparisons.

variable	d.f.	χ^2	p	order
$Total_1$	4	86.75	< 0.001	1 > 2 ≈ 4 > 0 > 3
$Total_2$	4	224.73	< 0.001	1 > 2 ≈ 4 > 3 > 0
$Total_{sum}$	4	190.90	< 0.001	1 > 2 ≈ 4 > 3 > 0
$Total_{together}$	3	44.878	< 0.001	3 > 1 ≈ 2 > 0

Table 6.6. Results of KW test on the indices of eggs dispersal for different oviposition substrates (0-4). Coding as for Table 6.5.

variable	d.f.	χ^2	p	order
I_1	4	53.23	< 0.001	4 > 1 ≈ 2 > 3 > 0
I_2	4	74.35	< 0.001	1 > 2 ≈ 4 > 3 > 0
J_{sum}	4	52.89	< 0.001	4 > 3 > 1 > 2 > 0
C_{ii}	4	57.43	< 0.001	3 > 4 > 0 ≈ 1 > 2
$J_{together}$	3	28.67	< 0.001	3 > 2 ≈ 1 > 0

Table 6.7. Results of Wilcoxon-signed rank test for matched pairs on egg totals ($Total_{independent}$, $Total_{together}$) and egg distributions ($J_{independent}$, and $J_{together}$) of two females that oviposited independently but egg numbers combined (ind) and corresponding oviposition of the same females together (tog).

Medium	parameter	N	Z	p	order
plain	total eggs	75	-6.33	< 0.001	ind > tog
	aggregation	76	-1.07	ns	
soaked	total eggs	28	-3.39	< 0.001	ind > tog
	aggregation	27	-2.55	0.011	ind > tog
dipped	total eggs	141	-1.02	ns	
	aggregation	140	-2.63	0.009	ind > tog
artificial	total eggs	32	-1.67	ns	
	aggregation	29	-2.09	0.036	tog > ind

Females tested for consistency, showed no significant changes in either egg totals from one day to the next (WSR test; Z = -0.48, p = ns, N = 60) or in the extent to which they aggregated them (WSR test; Z = -0.29, p = ns, N = 60). However, females showed consistency in egg totals, i.e. those that laid a large number of eggs on day 1 did so on day 2 (Spearman's rank correlation, $r_S = 0.48$, $p < 0.001$, N = 60) but this was not true for the degree of aggregation (Spearman's rank correlation, $r_S = 0.07$, p = ns, N = 58). There was no association between the parameters, $Total_1$ and I_1 or $Total_2$ and I_2, with non-significant Spearman's rank correlation coefficients for both comparisons.

Effect of substrate

The type of oviposition substrate used had clear and significant effects on the oviposition behaviour of females (Tables 6.5 and 6.6). Yeasted grape halves received most eggs from females 1 and 2, soaked ones being more popular than dipped. Plain grapes were less desirable as were food plugs of culture medium, plain grapes receiving the fewest eggs in total (Fig. 6.2). Strikingly, far more eggs were laid in total when oviposition by female 1 and 2 was on consecutive days than when they were in the arena together (Fig. 6.2). Further, when egg numbers of two females on day 1 were super-imposed ($Total_{independent}$), significantly more eggs were 'observed' than when the two flies were genuinely together. This was only true however, on plain and soaked grapes (Table 6.5).

Where there was a choice of dipped and plain grapes, dipped grapes were clearly preferred. Females laid significantly more eggs on dipped patches (mean day 1 = 19.20, SD = 12.98) than on plain halves (mean = 3.99, SD = 7.19) during trials (WSR test; Z = -7.04, $p < 0.001$, N = 120). Totals did not differ significantly from those in trials with dipped halves only (Table 6.7).

Egg distributions were also affected by medium type but effects were variable. Egg dispersal by females 1(I_1) and 2 (I_2) was clearly aggregated on all medium types, as was the combined distribution of eggs, J_{sum} (Fig. 6.3), although individuals showed great variation. Females aggregated least on plain grape halves (Table 6.6) with mean ranks for I_1, I_2 and J_{sum} being the lowest in the comparison. The behaviour on artificial medium was more complex. Both females generated less aggregated distributions on their own (I_1, I_2) compared to soaked and dipped grapes or indeed to the mixed set-up (Table 6.6), while the total degree of aggregation in the arena (J_{sum}) was clearly higher

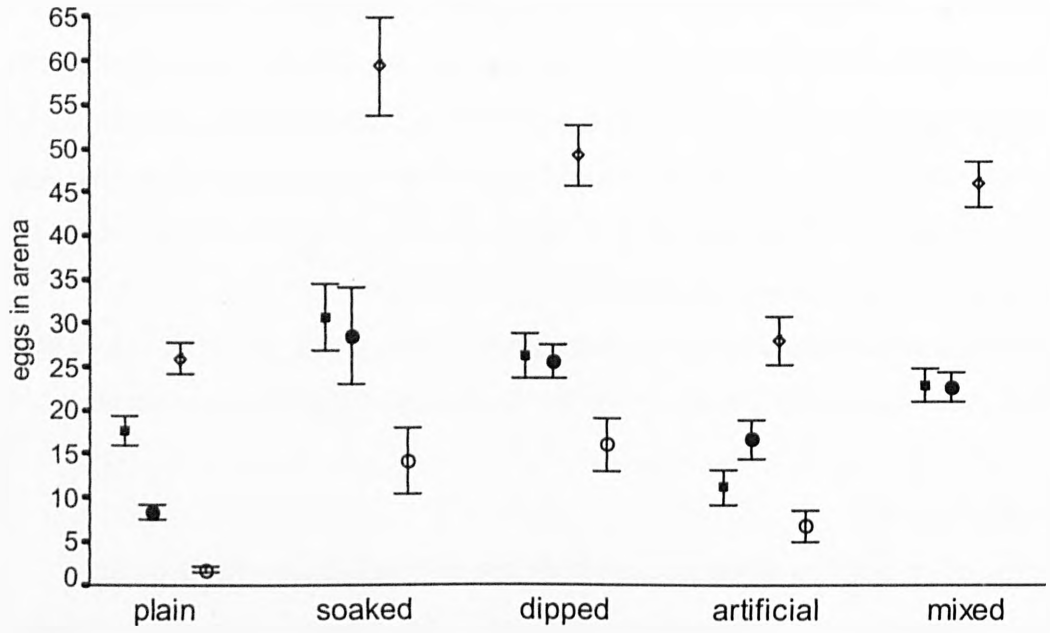

Fig. 6.2. Mean total eggs (± 95% C.I. based on s.e. mean) in one arena on different oviposition substrates. Symbols denote different totals: solid square = $Total_1$; solid circle = $Total_2$; open diamond = $Total_{sum}$ (eggs of ♀♀ *i* and *j* summed up); open circle = $Total_{together}$ (eggs laid by two flies simultaneously).

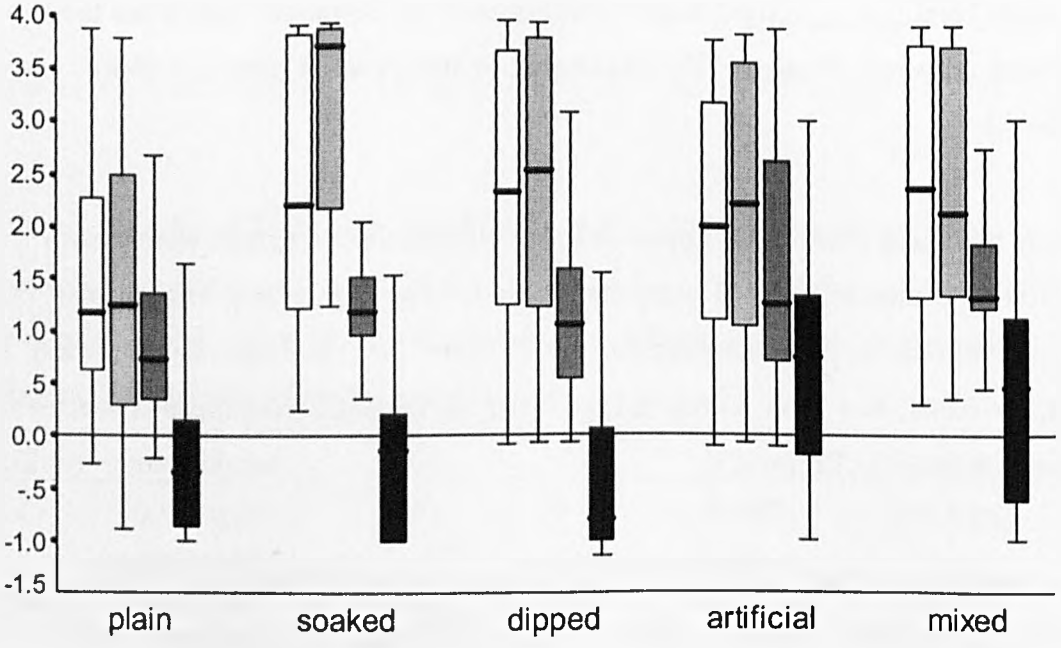

Fig. 6.3. Indices of dispersal generated by female *D. simulans* on different oviposition substrates. Box shading indicates index: white = I_1, light grey = I_2, dark grey = J_{sum}, black = C_{ij}. The reference line at 0 indicates a random distribution of eggs (*I* and *J*) or random association between eggs of ♀♀ *i* and *j* (C_{ij}).

Boxplot: boxes represent the inter-quartile range with the median (black bar); whiskers represent most of the remaining data except outliers and extreme observations (not displayed).

on artificial patches. Examining the degree of association between eggs of female 1 and 2, or, in other words, the degree of patch use overlap (C_{ij}) showed that while females on dipped, soaked and plain grapes generally avoided each other with median values of $C_{ij} < 0$ (Fig. 6.3), on artificial medium they clearly overlapped in patch use (median $C_{ij} > 0$).

In the set-up with a mix of plain and dipped grapes, indices of dispersal I_1, I_2 and J_{sum} were almost indistinguishable from those generated on dipped grapes only, but the association between eggs of female 1 and 2 was positive (Fig. 6.3). Comparing egg distributions generated by super-imposing patches of females 1 and 2 ($J_{independent}$) to those generated when these females oviposited together ($J_{together}$), $J_{independent}$ was significantly larger in paired sample comparisons than $J_{together}$ on all grape treatments while the reverse was true on artificial medium (Fig. 6.4, Table 6.7).

Totals ($Total_{all}$) and their corresponding aggregation indices (J_{all}) of 20 females selected randomly from one batch also differed between media (KW test for J_{all}; $\chi^2 = 10.69$, d.f. = 4, $p = 0.031$; but just non-significant for totals; $\chi^2 = 9.09$, d.f. = 4, $p = 0.059$; see Figs. 6.5 and 6.6). Comparison of simultaneous egg oviposition to that of super-imposing 20 random samples from day 1, indicates that females probably laid fewer eggs when in one arena simultaneously than in isolation. Eggs were still aggregated but very importantly, the degree of aggregation in arenas with 20 females was very much reduced (comparing Fig. 6.6 to Figs 6.3 and 6.4) and with the exception of mixed patches probably not significantly different from zero (Fig. 6.6). On day four, all four patches contained eggs, in all cases and on all media.

Changes in individual behaviour and associations between parameters

The behaviour of individuals in terms of total eggs deposited and distributions generated changed on some substrates but not on others (Table 6.8). On plain grapes, females laid consistently fewer eggs on day 2, i.e. when eggs of a conspecific female were present in the arena. The reverse was true for flies on artificial medium. The aggregation index differed between days only on soaked grapes, where females aggregated eggs significantly more on day 2 than on day 1. Testing associations between parameters of one female on the two consecutive days, egg output only

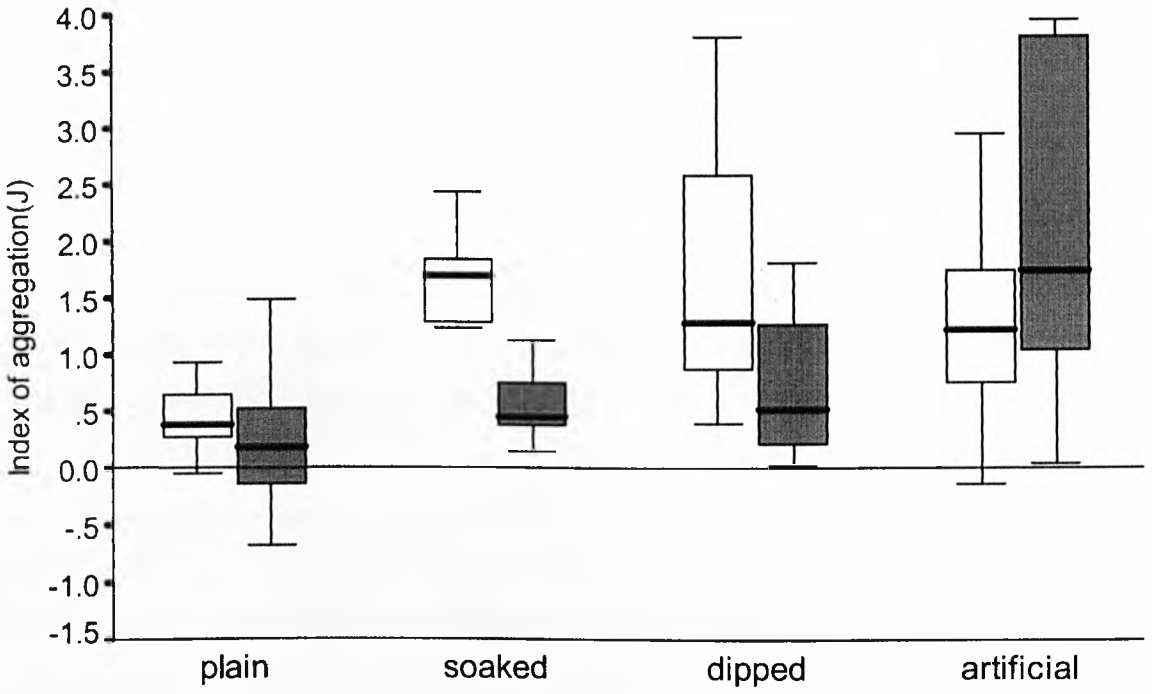

Fig. 6.4. The index of aggregation generated by eggs of two females independently but then combined ($J_{independent}$ = white box) and by the same females simultaneously ($J_{together}$ = grey box) on different oviposition substrates. The reference line at 0 indicates a random distribution of eggs.

Table 6.8. Results of WSR tests on the total eggs laid by individual females on the two first days of experiments ($Total_1$, $Total_2$) and their distribution (I_1, I_2) where eggs of a conspecific competitor are present on day 2.

medium	pair	N	Z	p	order
plain	totals	146	-5.61	< 0.001	$Total_1 > Total_2$
	aggregation	159	-0.30	ns	
soaked	totals	54	-0.74	ns	
	aggregation	54	-2.56	0.047	$I_2 > I_1$
dipped	totals	139	-0.62	ns	
	aggregation	128	-1.15	ns	
artificial	totals	64	-3.70	< 0.001	$Total_2 > Total_1$
	aggregation	64	-0.99	ns	
mixed	totals	120	-0.29	ns	
	aggregation	120	-0.86	ns	

remained consistent on dipped grapes (Spearman's rank correlation; $r_S = 0.39$, $p < 0.001$, $N = 139$). Aggregation indices I_1 and I_2 for females were never consistent.

The way egg numbers and distributions changed in one arena and between isolated and group scenarios also depended on substrate type but some patterns were consistent. Aggregation indices, I_1 and I_2, with the exception of mixed patches, never showed any significant positive or negative association. Nonsensical comparisons, e.g. eggs (totals or dispersal) of female 2 affecting female 1, never generated any significant associations. The extent of aggregation caused by individuals (I_1, I_2) always correlated positively with the total degree of aggregation (J_{sum}), i.e. the more individuals aggregated their eggs, the more their combined distribution was aggregated on day 2. Yet, this was not true for egg distributions in isolation compared to those in 2-individual set-ups ($J_{together}$, day 3); there were no significant associations between this parameter and I_1 or I_2 . Otherwise, patterns varied according to substrate type; Table 6.9 summarises results of comparisons.

Most interactions between variables were observed on plain grapes (Table 6.9). Eggs of female 1 affected the behaviour of female 2; the second fly laid fewer eggs but aggregated them more, the more eggs were already present in the arena. If female 1's eggs were very aggregated, the second female laid fewer eggs. Adding more eggs on day 2 lead to a decrease in the total degree of aggregation in the arena while the more aggregated individual egg distributions and the resulting aggregated total distribution (I_1,I_2 and J_{sum}) were accompanied by a decrease in patch use overlap (C_{ij}).

On soaked grapes, few significant interactions were observed except for the decrease in overlap associated with higher egg aggregation by fly 1 (though not fly 2).

Responses on dipped grapes were different to those obtained for plain ones. Female 2 responded to higher egg numbers of female 1 by adding more eggs, the reverse of the observation on plain grapes. Overlap however, was again reduced if individual aggregation was high and adding more eggs on day 2 decreased the total degree of aggregation in the arena.

On artificial medium, higher aggregation of eggs by female 1 corresponded to fewer eggs laid by female 2 (and hence fewer eggs in total) and fewer eggs were also found if

Table 6.9. Results of Spearman's rank correlations between the different parameters on the five oviposition substrate set-ups. Associations that were never significant are not listed although all were tested.

variable combination	resource type				
	plain	**soaked**	**dipped**	**artificial**	**mixed**
$Total_1$ vs. $Total_2$	r_S = - 0.18 p = 0.026 N = 158	ns	r_S = 0.21 p = 0.013 N = 133	ns	ns
$Total_1$ vs. I_2	r_S = -0.18 p = 0.031 N = 148	ns	ns	ns	ns
I_1 vs. $Total_2$	r_S = -0.18 p = 0.024 N = 155	ns	ns	r_S = -0.38 p = 0.002 N = 64	ns
I_1 vs. C_{ii}	r_S = -0.295 p < 0.001 N = 145	r_S = -0.17 p < 0.001 N = 56	r_S = -0.27 p = 0.002 N = 133	ns	ns
I_1 vs. $Total_{sum}$	ns	ns	ns	r_S = -0.43 p < 0.001 N = 64	ns
$Total_2$ vs. J_{sum}	r_S = -0.31 p < 0.001 N = 155	ns	r_S = -0.24 p = 0.005 N = 139	ns	ns
I_2 vs. $Total_{sum}$	ns	ns	ns	r_S = -0.26 p = 0.039 N = 66	ns
I_2 vs. C_{ii}	r_S = -0.19 p = 0.026 N = 145	ns	r_S = -0.28 p = 0.010 N = 133	r_S = 0.28 p = 0.027 N = 64	ns
J_{sum} vs. C_{ii}	r_S = -0.18 p = 0.029 N = 145	ns	ns	r_S = 0.77 p < 0.001 N = 66	r_S = 0.69 p < 0.001 N = 120

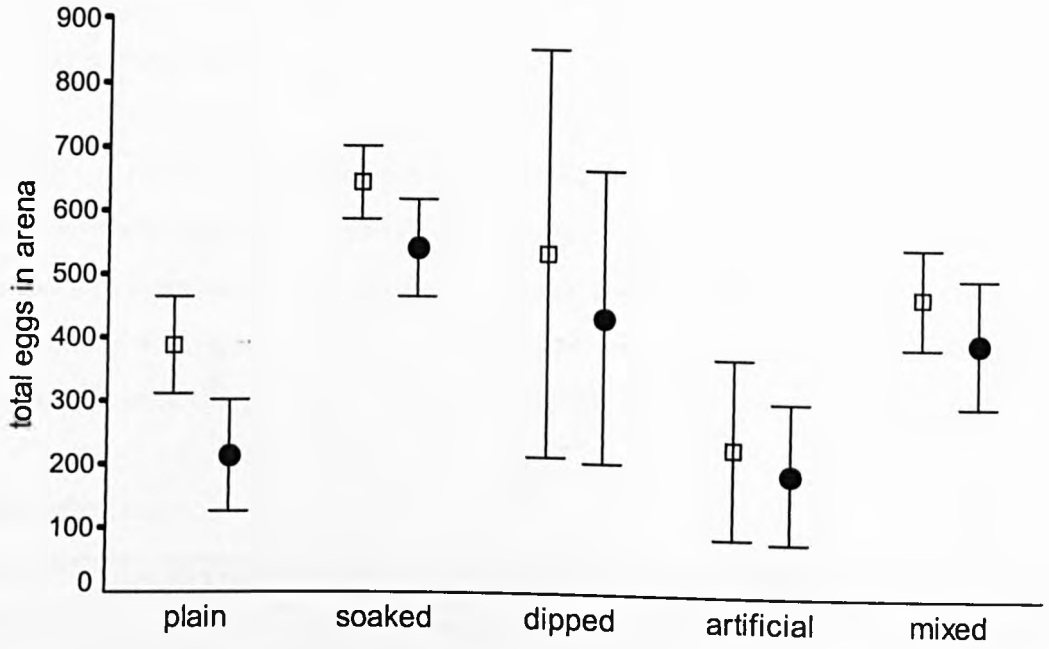

Fig. 6.5. Total eggs in arena (± 95% C.I.) of groups of 20 *D. simulans* either in individual arenas (day 1) then combined ($Total_{20}$ = open square) or simultaneously in one arena ($Total_{all}$ = solid circle).

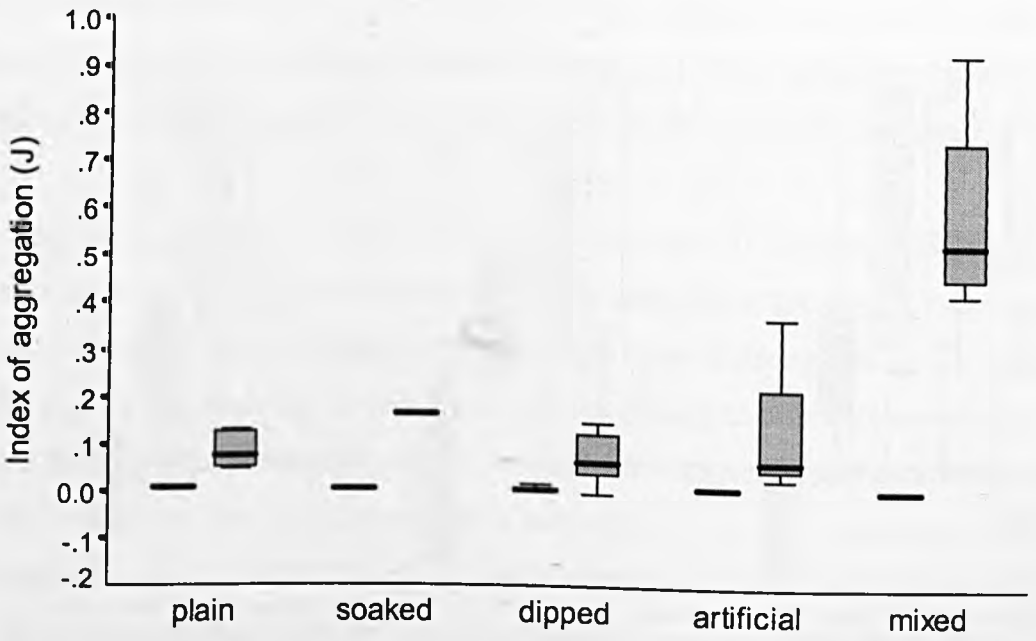

Fig. 6.6. Indices of aggregation of eggs produced by groups of 20 *D. simulans* either in separate arenas (day 1) then combined (J_{20} = white boxes, only medians visible) or simultaneously in one arena (J_{all} = grey box). **Note the difference in scale on the y-axis compared to Figs 6.3, 6.4 and 6.8.**

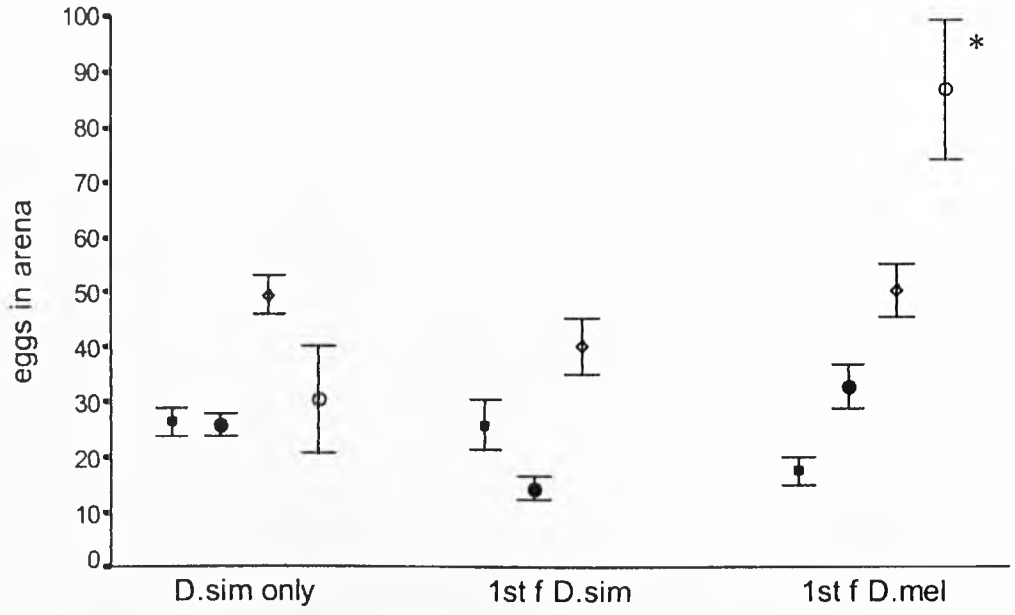

Fig. 6.7. Total eggs in arena (± 95% C.I.) of females in different set-ups. D.sim only = ♀ *i* and *j* are *D. simulans*; 1st f D.sim = ♀ *i* is *D. simulans*, ♀ *j* is *D. melanogaster*, 1st f D.mel = ♀ *i* is *D. melanogaster* and *j* is *D. simulans*. Oviposition substrates were dipped grapes only. Symbols denote different egg totals: solid square = $Total_1$, solid circle = $Total_2$, open diamond = $Total_{sum}$, open circle = $Total_{together}$. * = total is the same for both mixed species set-ups.

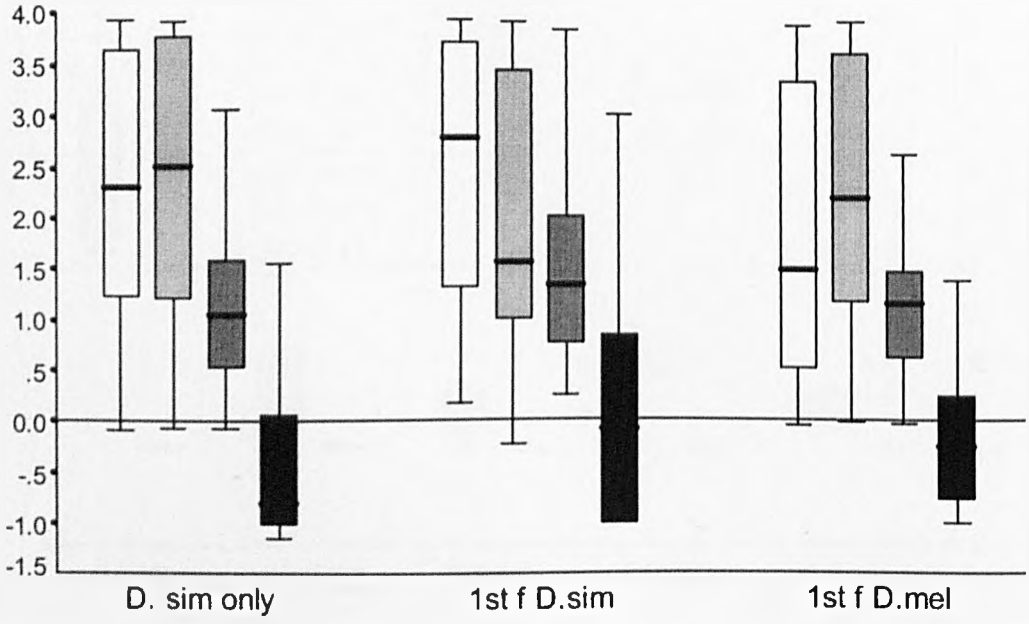

Fig. 6.8. Indices of dispersal generated by female *Drosophila* in different set-ups. x-axis labels as above (Fig. 6.7). Oviposition substrates were dipped grapes only. Box shading indicates index type: white = I_1, light grey = I_2, dark grey = J_{sum}, black = C_{ij}. The reference line at 0 indicates a random distribution of eggs (*I* and *J*) or random association between eggs of female *i* and *j* (C_{ij}).

I_2 was very high. In contrast to results obtained on the grapes, increases in I_2 and J_{sum} were associated with more patch use overlap (C_{ij}).

Finally, interactions on mixed patches were very limited. As on culture medium, higher levels of aggregation generated by the two females (J_{sum}) occurred if patch use overlap was high but this was the only resource set-up where more aggregated egg distributions of female 1 also increased aggregation in female 2 (not listed in Table; Spearman's rank correlation; $r_S = 0.26$, $p = 0.005$, $N = 120$).

Effect of species

Egg totals for *D. simulans* on day 1 were indistinguishable from those of *D. simulans* in the one species set-up but *D. melanogaster* laid significantly fewer eggs (Table 6.10, Fig. 6.7). On day 2 however, with the eggs of the first female present, *D. simulans* in the two species system laid significantly more eggs than did *D. simulans* in single species system while *D. melanogaster* still laid fewer (also supported by significant WSR test; $Z = -4.35$, $p < 0.001$, $N = 61$, for *D. simulans* = first ♀). This also led to a significant difference in total eggs (female 1+2) in arenas where *D. melanogaster* were the first females (Table 6.10, Fig. 6.7). On day 3, *D. simulans* and *D. melanogaster* together produced the highest egg numbers ($Total_{together}$) compared to the single species set-up on any medium type (KW test; d.f. = 5, $\chi^2 = 22.09$, $p < 0.001$, see also Fig. 6.7).

D. simulans aggregated their eggs significantly more than *D. melanogaster* in either scenario (I_1, I_2) although the patterns generated by both flies together (J_{sum}) were too similar to the single species results to produce significant differences (Fig. 6.8, Table 6.10). Aggregation patterns for individuals did not change for either *D. simulans* or *D. melanogaster* due to the presence of a competitors eggs (non-significant WSR test results). Patch use overlap (C_{ij}) was higher for the mixed species scenario than for *D. simulans* only. The index of aggregation produced by the two females, one of each species, together on day 3, did not differ significantly from that generated by two *D. simulans* (KW test; d.f. = 1, $\chi^2 = 3.07$, p = ns).

Table 6.10. KW tests on egg totals in arenas and their distributions for different species assemblies: 1 = *D. simulans* only (first and second ♀); 2 = first ♀ *D. simulans*, second ♀ *D. melanogaster*, 3 = first ♀ *D. melanogaster*, second ♀ *D. simulans*.

≈ = not significant in pairwise MWU comparison.

variable	**d.f.**	χ^2	**p**	**order**
$Total_1$	2	10.29	0.005	$1 \approx 2 > 3$
$Total_2$	2	57.43	< 0.001	$3 > 1 > 2$
$Total_{sum}$	2	11.01	0.004	$3 \approx 1 > 2$
I_1	2	7.36	0.025	$2 \approx 1 > 3$
I_2	2	9.29	0.009	$1 \approx 2 > 3$
J_{sum}	2	3.62	ns	
C_{ij}	2	10.45	0.005	$2 \approx 3 > 1$

Discussion

The oviposition behaviour in *Drosophila* is clearly complex and influenced by a variety of factors. In these experiments, I investigated several possible factors but some of them had no effects. These are discussed first.

Males

Females of several *Drosophila* species are known to respond to aggregation pheromones, emitted by both female and male conspecifics (e.g. Jallon *et al.* 1981). Female responses are measured in olfactometers and have been demonstrated for *D. virilis* (Bartelt & Jackson 1984), *D. melanogaster* (Bartelt *et al.* 1985) and *D. simulans* (Schaner *et al.* 1987). Although it is suggested that *D. simulans* are attracted to aggregation pheromones in olfactometers, they generally must be deprived of food for at least two hours before they show any response to the volatile chemicals (Baertelt & Jackson 1984; Schaner *et al.* 1987). Aggregation pheromones may serve as a vital cue for finding suitable feeding sites during periods of starvation (see Spieth 1974) but my results suggest that they are of little importance in well-fed flies. Alternatively, the nature of the chemical may be such that it has no conditioning effect on the oviposition sites (i.e. it may evaporate too quickly) and was therefore ineffective in my experiments. Actual male presence in arenas however, also had no effect on the spatial distribution patterns of oviposition. There is some indication that male presence may disturb egg-laying because females laid fewer eggs in trials with males. The negative effect of male presence on both survival and egg production in *Drosophila* is well known. Partridge *et al.* (1986) demonstrated it in *D. melanogaster* and suggested that males may depress female survival and oviposition, perhaps by contamination of food, harassment of females or as a physiological consequence of mating itself. It should be emphasised however, that the differences in egg totals in my experiments could also have been due to a batch effect, i.e. they may have been caused by differences in the quality of grapes during the trials with male presence. Further replication (i.e. more than one batch) could have eliminated this possibility; the effect may be genuine since it has been observed elsewhere (Partridge *et al.* 1986). Aggregation pheromones may be more important if females are virgin (or are searching to re-mate), and their possible role in the context of aggregated oviposition is not

disproven by this study. In mated and well-fed females of *D. simulans* however, males appear to have little effect.

Pre-experimental adult density

Adult population density is known to influence oviposition behaviour in several insect groups. Hymenopteran parasitoids, for example, adjust their clutch sizes (and hence the degree of aggregation) in response to the adult female density experienced immediately before oviposition site selection; the direction of the adjustment depends on the form of larval competition, i.e. contest versus scramble, prevalent among the larval offspring (Visser & Rosenheim 1998). For the gregarious parasitoid *Aphareta minuta*, with scramble competition among their larvae, clutch size decreases with increasing adult density (Visser 1996). Density-dependent oviposition is also known in Lepidoptera (Binder & Robbins 1996), Dictyoptera (Gordon *et al.* 1994) and Coleoptera (McNeill *et al.* 1998). During these experiments there was little evidence that *D. simulans* oviposition behaviour was influenced by the pre-experimental density of other adult females apart from the near-significant, slight increase in aggregation for females from high density conditions. The evidence that adult density during oviposition is important was much more convincing; egg numbers and egg distribution patterns were affected by interaction with other females. On the more natural substrates (all grape types), females laid fewer eggs in group situations than they did individually and the response was more marked, the higher the adult density. Additionally, egg distributions were less aggregated when two females were in one arena together than when eggs had been deposited in isolation and sequentially. Yet, once the group size increased to 20, eggs became more aggregated in simultaneous arrangements than if numbers for 20 females in isolation were superimposed. Other, density-dependent changes in behaviour are discussed after an examination of the effects of oviposition substrate.

Substrate

The quality of the oviposition substrate, i.e. the characteristics of the breeding sites, had very clear impacts on the behaviour of females. The preference for yeasted grapes is very apparent in these experiments. They received far more eggs than plain grapes which was true for totals on all days, i.e. of individuals and for groups. Although this may be due to facilitated ease of detection, i.e. yeast metabolic activity giving rise to odour cues, the results of the previous chapter suggested that females largely 'decide' about the quality of the resource after arriving at a patch (see also Shorrocks & Bingley

1990). The probability of leaving a resource patch once it is located is highly variable, is likely to depend on its characteristics and is clearly not random. It appears that on resources that are of higher quality, females are less likely to leave once they have located them; yeasted grapes clearly represent such high quality resources (see also Chapter 3). As a direct result of remaining on a patch and continuing to oviposit, the degree of aggregation is significantly larger than on poorer quality resource, for aggregation of individuals' eggs and that of a group. I hypothesise that on poorer quality resources (i.e. plain grapes), the probability of leaving a patch is higher as females need to continue to search for a better resource. In the experiments, this is supported by total egg numbers, the degree of aggregation and the number of patches containing eggs after day 1. While larval survival (investigated in Chapter 3) reflects the choice differences for dipped and plain grapes, i.e. survival probability is higher on dipped than on plain grapes as are egg totals and aggregation, females appear to 'misjudge' yeast-soaked grapes. Most eggs were deposited on soaked grapes and aggregation was highest but Chapter 3 clearly showed that they were a poor bet in terms of offspring survival. This may be explained because *Drosophila* respond to volatile cues such as yeast metabolic products (ethanol or acetic acid), during their search for oviposition sites (e.g. Jaenike 1982). At the same time *D. simulans* larvae are unable to tolerate high concentrations of ethanol (e.g. Parsons & Spence 1981). In the field, very high alcohol concentrations are less likely as 'yeast-soaking' does not occur which may explain why *D. simulans* females respond to the cue (see also Richmond & Gerking 1979) but cannot discriminate for intensity (but see Jaenike 1982).

Culture medium is peculiar in that it is well adapted to the needs of laboratory fly stocks but nevertheless did not generate high egg numbers. This is almost certainly due to the surface texture of agar-based food where oviposition can be (and often is) stimulated by providing irregularities in the surface structure, either artificially or by the presence of other eggs (Atkinson 1983). Here, surfaces were left smooth and shiny (and convex, see Ruiz-Dubreuil *et al.* 1994). Results for aggregation indices support the findings of Atkinson (1983): while I_1 and I_2 ranked lower in the comparison with yeasted grapes, combined aggregation J_{sum} and $J_{together}$ (i.e. aggregation for two females consecutively or together) and for 20 females ranked higher, as did overlap (C_{ij}). Females on artificial medium thus prefer to oviposit on sites that contain eggs already. Probably as a result of added surface irregularity and increased female egg load due to having laid little on day 1, egg totals on day 2 were higher than on day 1.

The substrate clearly affected the way individual behaviour changed over the days of the experiment and in response to changing conditions in the arena but it is the more general patterns that are most interesting. The extent of aggregation caused by the first female (i.e. her clutch sizes) had no direct or linear effects on that caused by female 2, on all but artificial medium. Yet, eggs present affected female behaviour as, unlike the experiments where flies were exposed to fresh grapes on day 1 and 2 (consistency; see also Chapter 1), totals were no longer consistent for the two days (except for dipped grapes). The direction of change in egg totals however, was not consistent but depended on substrate type. There were general patterns, too, when comparing oviposition in isolation to combined oviposition (consecutive or simultaneous). Individuals with higher aggregation indices on their own also had higher indices combined (J_{sum}) but not when oviposition was simultaneous ($J_{together}$ or J_{all}), indicating again that female behaviour changes in response to the presence of other females. In addition, increases in total aggregation are not due to gregariousness but instead are a result of larger individual clutch sizes, clearly supported by the negative C_{ij} values. In other words, female *D. simulans* produce aggregated egg distributions despite avoiding each others' oviposition sites rather than because of gregariousness. Only when all or nearly all patches were used already (low I_l), did overlap increase, i.e. females avoided each other if possible but utilised used sites when no choice was available. This is also supported by the general decrease in aggregation observed in arenas from day 1 to day 2 and for females ovipositing in pairs or at densities of 20, where distributions across patches became near random. It is important to note that females appeared not to discriminate between eggs of conspecifics or their own, as no differences were detected (use of same female on day 1 and 2).

Two experimental set-ups generated results that differed from the above. On artificial substrate, females responded to increased aggregation by female 1, by also aggregating eggs more, a direct result of the positive overlap. Here, increase in aggregation by females 1 and 2 is due to gregariousness rather than larger individual clutches and this has important implications for other aggregation work. The analysis showed that females that aggregate individually produce more aggregated distributions in total which supports the findings of del Solar & Ruiz (1992). Yet, artificial medium represents an exception because on natural resource types this appears to be more a function of large individual clutches than of gregarious association in breeding site use between the two females. In a further study Ruiz-Dubreuil *et al.* (1994) addressed the

issue of natural versus artificial substrate. They found that egg numbers were far higher on grapes (they used halves seeded with live yeast suspension, i.e. 'dipped') but that aggregation was unaffected and differences between lines selected for high and low aggregation were maintained. In fact, eggs per female per trial, if calculated, were still surprisingly low in their experiments (see Ruiz-Dubreuil *et al.* 1994). This is a consistent observation in all their studies (although they usually use artificial medium) and may indicate that there is an important shift in behaviour, not only the more females are present in a closed arena but also, surprisingly, as more patches are available. The equivalent mean egg numbers for 20 females in my experiments suggest comparatively only a slight (though still significant) decrease in egg numbers in groups relative to isolated situations (22.5 eggs per female per 24 hours for 20 females when together and 27.5 eggs per female per 24 hours when alone). It is difficult to see how this discrepancy can be explained by the availability of patches since there were only four in my experiments compared to 20 or 25 in other studies. It remains questionable whether del Solar and co-workers are not selecting for a few females that lay large clutches and contribute largely to the egg numbers found while others lay small clutches or no eggs at all (see also Chapter 5).

Del Solar (1998) recently published a further study in which he tracked the behaviour of individuals in group situations more closely by using genetic markers. The protocol of the study is excellent but findings are restricted to the use of artificial medium and the link between clutch size and gregariousness is little explored. In his study aggregation (of eggs of the group) in the population cages increased over the first two days of the experiment, leveled off and then decreased towards 9 days. A number of females never used more than one tube. From the findings of my study, I would argue that the increase in aggregation is due to the modification of the surface texture (caused by the insertion of eggs, larval activity) and the varying preference of individuals for such surface irregularities. Yet, it is unclear which behavioural traits are important in producing aggregation on artificial medium; variation between individuals may be explained by genetic variability in the tendency for egg insertion behaviour (see previous chapter, Albornoz & Domínguez 1987), preference for soft versus hard medium (Takamura 1980) or by differences in the ability of individuals to detect sites previously used by other females (e.g. response to pheromones, visual cues). Ruiz-Dubreuil *et al.* (1994) also found differences in locomotory activity patterns between females of strains selected for high and low gregariousness (on artificial medium).

Low-line females were more active, were more dispersed as adults across patches and produced less aggregated egg distributions. Yet, this does not resolve the problem of exactly which traits females were selected for in the first place although activity patterns may offer some explanation. From the results of my study it is clear that oviposition behaviour is very different on more natural substrates where avoidance of previously used oviposition sites rather than gregariousness is the norm while aggregated egg distributions are generated to a similar extent as on artificial substrates.

Decreasing the number of high quality patches also had interesting effects. Results for egg totals in mixed patch scenarios suggest that, although fewer dipped patches were available, flies had no problems finding and using them. Totals and aggregation patterns were similar to those from dipped grapes only but patch use overlap increased. This is almost certainly because two out of four patches were of poorer quality and females so strongly preferred laying on yeasted grapes that avoidance was no longer important. The numbers generated suggest that this is not a bad strategy; although combined oviposition by the two flies produced much higher egg numbers on the dipped than on the plain grapes, we know from chapter 3 that survival on dipped grapes is much higher. The link between oviposition site choice, clutch size and consequences for survival and other fitness parameters will be explored more closely in the next chapter.

The general conclusion is that flies appear to be able to make 'judgements' about the quality of oviposition sites and can be influenced by egg densities already present on patches. The density-dependence of oviposition and aggregation has important implications which will be discussed later. To distinguish the response to either resource quality or egg presence is difficult in these experiments. On plain grapes for example, females laid fewer eggs on day 2 than on day 1; this may be due to the overall poor quality and the presence of eggs but could also be due to a deterioration of the resource from one day to the next (e.g. mould beginning to grow, drying out of surface). It is nevertheless clear from the results that presence of eggs and presence of conspecifics affect the behaviour; the relationship between females is competitive. In her assessment, a female does not however, discriminate between her own eggs or those of a conspecific competitor. Females will avoid laying on patches that already contain a number of eggs thus generating distributions that can still be aggregated but are not the result of gregarious behaviour. The outcome is different however, if the surface texture

(or possibly other resource properties) requires the co-operation of females, or, if the quality of patches available is variable. The implications of these results for aggregation theory and the evolution of aggregation will be discussed in more detail in the final chapter, the general conclusion.

Hetero-specific interactions

The two sibling species differ slightly in their oviposition behaviour. *Drosophila simulans* lay more eggs than *D. melanogaster* and also aggregate eggs to a higher degree on the resource type tested (dipped grapes). Both species are known to breed in vineyards where grapes represent a natural oviposition substrate (e.g. McKenzie 1974) and females of either species oviposited on grape segments added to their culture vials readily enough prior to experiments. Chess *et al.* (1990) also demonstrated, although they used artificial medium, that *D. simulans* laid more eggs than *D. melanogaster* and were, in fact, more fecund. Further, they showed that each species produced more eggs when they were tested together than when they were alone which is clearly supported by my results. When the first female in the arena was *D. melanogaster*, *D. simulans* responded by laying more eggs than they did in the one-species scenario; *D. melanogaster* showed no such response. This effect was also observed when *D. melanogaster* and *D. simulans* oviposited simultaneously in the two individuals situation. The egg totals in the two species situation suggest that although *D. melanogaster* did not respond to the presence of eggs of *D. simulans*, they did respond to the presence of adult female *D. simulans* by increasing oviposition output. Although *D. simulans* are more fecund they do not out-compete *D. melanogaster* in regions where they co-occur, or indeed, in population cage experiments in the laboratory (Chess *et al.* 1990). In fact, the opposite is true and *D. simulans* are frequently excluded if *D. melanogaster* are present (e.g. Hedrick 1972). The precise mechanism of these competitive differences is poorly understood but *D. melanogaster* larvae appear to be competitively superior to *D. simulans* since they are not adversely affected by high densities of *D. simulans* while the reverse is true for *D. simulans* larvae (Atkinson 1979).

It is important to note that the total degree of aggregation in the two-species situation is indistinguishable from that generated by conspecific competitors, both in consecutive and simultaneous oviposition. The measure of overlap in patch use suggests surprisingly that *D. melanogaster* and *D. simulans* generate egg distributions that are

more associated with each other than do *D. simulans* alone. Yet, it is important to stress that the median values were close to random distributions ($C_{ij} = 0$), i.e. females showed no preference to oviposit on the same patches either. Chess *et al.* (1990) suggest that use of the same patches is not the only way of looking at patch overlap, but rather that the distribution of eggs within the patch may be important. According to their data, *D. melanogaster* prefer to lay eggs near the edges of patches while *D. simulans* lay more in the centre. I noticed no such divergence in behaviour and it is questionable how important this would be since larvae begin to move around the patch as soon as they have hatched. The shift in the degree of patch overlap from avoidance between conspecifics to random associations in hetero-specific scenarios is interesting. It could indicate that it is more important to avoid conspecifics because competition for resources is more scramble than competition with other species. One of the major underlying assumptions for aggregation to promote coexistence is that closely related species distributed their eggs randomly with respect to each other and hence, that aggregation of eggs of the two species is independent (e.g. Shorrocks & Rosewell 1987; Shorrocks 1990) and such independence is clearly supported by the findings of this study. Sevenster (1996) and Sevenster & Van Alphen (1996) however, found that in the field there are associations between species that are consistent from year to year but they concluded that aggregation could nevertheless explain coexistence in their neotropical *Drosophila* community. Positive associations between species may therefore represent less of a problem in analysing the occurrence of coexistence and its stability in the field than large and variable clutch sizes (see also Sevenster 1996).

Chapter 7 - Grape sugar concentration and oviposition site choice: the implications for fitness

Summary

Female *D. simulans* oviposition choices were investigated in response to varying sugar concentrations in grapes, either treated with yeast or left untreated. Consequences of female choices for offspring survival and fitness were tested, using two protocols: larval densities either reflected oviposition patterns of females or were experimentally manipulated. Females laid fewer eggs on grapes with high sugar content but only if yeast was present. Higher sugar content increased survivorship and adult body size. Female choices (clutch sizes and their distributions) reflected the differences of oviposition sites in terms of their suitability as breeding substrates for larvae. Density-dependent effects indicate however, that oviposition site choice is likely to be a problem of optimal foraging strategy.

Introduction

In the previous Chapter, I demonstrated that egg numbers and egg distributions alter according to substrate type. Additionally, results from Chapter 3 indicate that these variations reflect on the relative quality of the resource in terms of the number of developing larvae they can support. A clear difference between yeasted and non-yeasted grapes was detected. Within a substrate type there was, however, still a large degree of variation in the response of different individuals, indicating that factors other than yeasts may be important in influencing the oviposition choices of female *D. simulans*. Many physical factors have been implicated (discussed in Chapter 4) but only some of them may be indicative of substrate quality, e.g. colour (see Grossfield 1978) or volatile chemicals (e.g. Jaenike 1982). It is possible, too, that the presence of conspecifics (eggs or larvae) to which females undoubtedly respond (see previous Chapter and Chapter 4) could be indicative of quality if the first female that chooses the resource responds largely to qualitative differences. Since the differences in oviposition choices have been recorded on grapes which are apparently similar, including size and wound size (see Chapter 4), this experiment aimed to reveal whether sugar concentration differences among grapes might be a factor influencing oviposition site choice and larval fitness.

Although sugar may be important, it is likely that a large combinations of factors make a resource a good choice for oviposition, the relative influences of which are hard to resolve. I therefore wanted to investigate whether the way females distribute their eggs and the patches they choose reflect differences in survival or fitness of their offspring. While the results of Chapter 3 and 6 strongly suggest that there is a link, a more conclusive experiment was desirable to test this directly. This was attempted in two ways: consequences of oviposition choices were investigated with as little manipulation of the resource and female as possible, and they were tested by a much more careful control of experimental conditions. The latter facilitated data analysis and allowed more powerful conclusions.

Materials & Methods

Recording oviposition choices

Flies used in these experiments were *D. simulans* from the wild strain collected in Zimbabwe, Africa. The stock had been maintained on standard *Drosophila* medium (see Appendix) for about three years. The basic procedure followed the protocol of the previous chapter. Females were isolated on their day of eclosion, using CO_2 as an anaesthetic, and kept with two males on standard medium in standard glass vials (25 x 75 mm). Two days prior to trials, pieces of grape were added to the vials to accustom females to this oviposition substrate. Trials involved releasing individual seven-day old females into an experimental arena (17 x 11.5 x 5 cm), containing four glass vials (25 x 75 mm), each with a grape half. Grapes were a Spanish seedless variety, frozen on the day of purchase and defrosted in tepid water prior to experiments; four different bunches were used. Grapes in one trial were the four halves of two split grapes whose sugar concentration was measured before placing them into the arena. A drop of grape juice from each half was squeezed into a Pulfrich refractometer (range 0-30%) to record sugar concentration. Calibration of measurements against solutions of known sucrose concentration had shown the apparatus to be fairly accurate with a standard error (mean) in the region of 0.11%, increasing slightly for solutions of $< 12\%$ and $> 26\%$. After measurement, grape halves were placed into the vials, filled with 12.5 g of moist sand, cut side exposed either untreated (plain) or dipped in 1% yeast solution (*Saccharomyces cerevisiae*) (dipped). Vials were placed, equally spaced, in one line in random order. Once the female had been released, arenas were left undisturbed in a cooled incubator at $22.5 \pm 0.5°C$ continuously illuminated with a light source

perpendicular to the line of vials. After 24 hours, females were removed and egg numbers per grape half were counted under a binocular microscope (using a cold light source). The distribution of eggs was quantified using the index of aggregation, *I* (see Chapter 4).

Investigating fitness

Two separate approaches were taken to test how the oviposition choice of a female would affect the survival and size of her offspring:

a) From the above trials, all grape halves containing eggs were placed into clean glass vials (25 x 75 mm); vials were stoppered with foam bungs and incubated at 22.5 ± 0.5°C until progeny emerged. The emerged adults were killed in 75% ethanol solution and their wing lengths measured from the anterior cross vein to the distal end of the 3rd longitudinal vein (see also Chapter 3). Grape halves without eggs were discarded.

b) From the above trials, 40 arenas (20 of each substrate type) were chosen randomly, five from each batch using a different bunch of grapes. A very thin top layer containing eggs or early first instar larvae was sliced off from grape halves that had been oviposited on during trials, using a sharp scalpel blade. Grapes that had not been oviposited on were treated in exactly the same way. If grapes had been dipped, they were again dipped into 1% yeast solution. Ten first-instar larvae collected from the stock culture (same procedure as in Chapter 3) were transferred onto each of the four grape halves of one arena. Grape halves were placed into clean glass vials (25 x 75 mm), stoppered with a foam bung and incubated at 22.5 ± 1 °C until adults emerged. Adults were killed in 75% ethanol solution and their wing lengths measured (see above).

Levels of replication

Ignoring trials in which no eggs were laid, a total of 281 valid trials remained for analysis: 142 arenas using plain and 139 arenas using dipped grapes. Of these, 241 sets of four halves were assigned to protocol a) while the remaining 40 were chosen for protocol b).

Statistical analysis

The design of the experiment allowed investigation of a number of factors. Before splitting the data into sets for protocol a) and b), the effect of substrate (plain or dipped

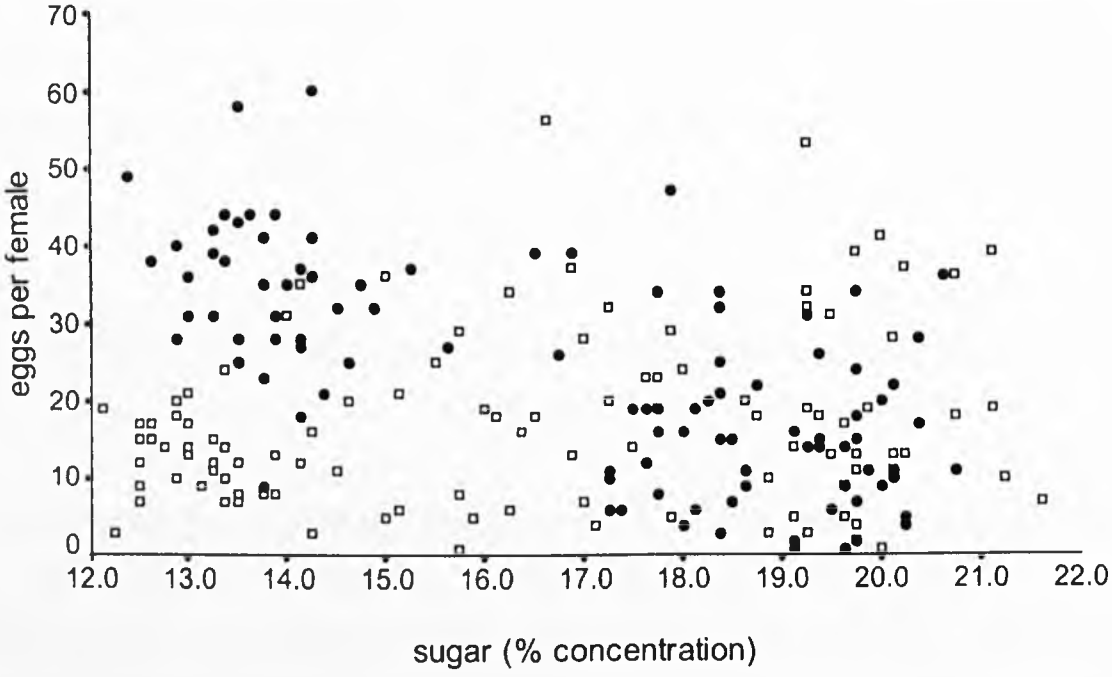

Fig. 7.1. The total eggs laid by female *D. simulans* over 24 hours during trials with differing mean sugar concentration in four grape halves. Open squares = plain grapes; solid circles = yeast-dipped grapes.

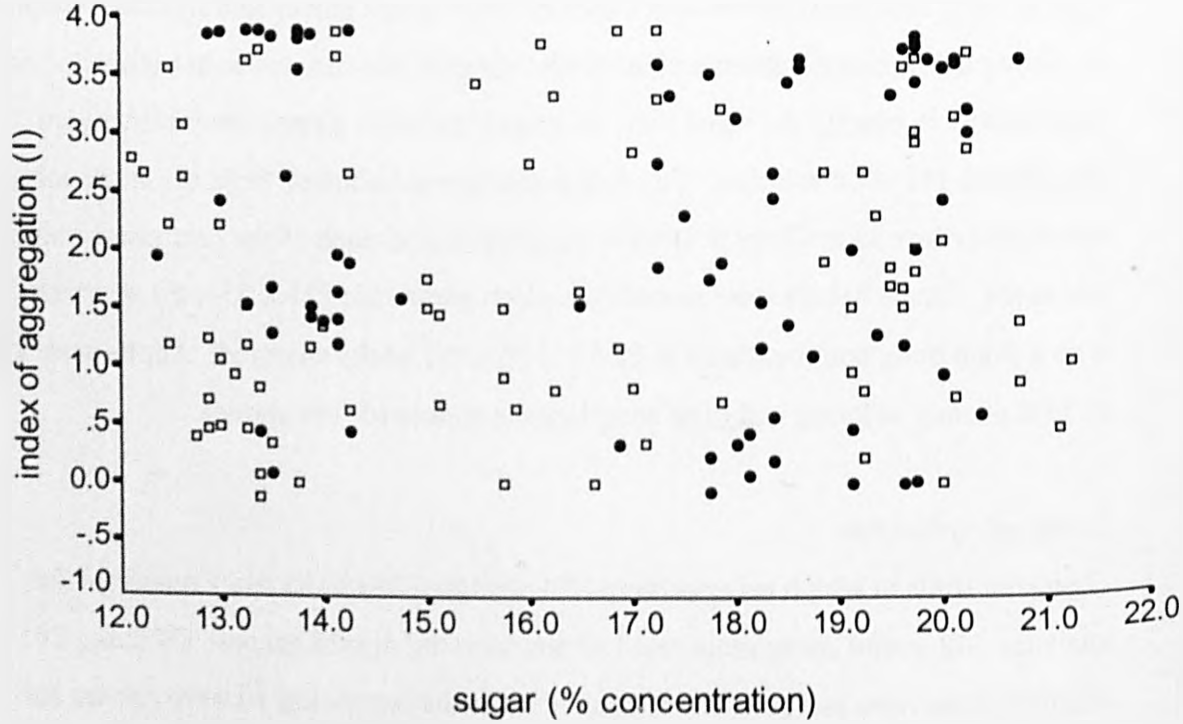

Fig. 7.2. Index of aggregation (*I*) of female *D. simulans* during trials with differing mean sugar concentrations in four grape halves. Open squares = plain grape; solid circles = dipped grapes.

grapes) and of sugar concentration on egg numbers and their distribution was tested. Since both egg totals and distributions are not normally distributed, the effects of substrate were tested using Mann Whitney U tests (MWU), while associations between parameters and sugar concentrations were investigated using Spearman's rank correlation (SRC) separately for each substrate type.

Further, the effects of substrate, sugar, egg numbers (density) and aggregation on fitness (survival and size) were tested on the subsets of data from protocols a) and b). Survival data from a) showed the same problems already discussed in Chapter 3; they are not normally distributed. The effects of substrate were analysed using a MWU test, SRC was carried out for the effects of sugar concentration and aggregation. To facilitate analysing the effects of initial egg numbers (density), they were subdivided into density classes (see also Chapter 3): class 1 = 1-2 larvae, class 2 = 3- 8 larvae, class 3 = 9-16 larvae, class 4 = 17-24 larvae, class 5 = 25-38 larvae and class 6 = 39 or more larvae. Differences in survival of density classes were analysed using Kruskal Wallis test (KW). Wing lengths were analysed using parametric 3-way analysis of covariance (ANCOVA) with sex, substrate and density as main effects and sugar concentration as the covariate; the effect of aggregation was tested using SRC. For protocol b), the number of larvae per grape half (and hence density) was fixed but a further factor included in the analysis was whether or not a grape had been chosen for oviposition during the basic procedure. Both survival and wing lengths conformed to normal distributions. Survival was analysed using 2-way ANCOVA, again with sugar as the covariate (main effects = substrate and whether chosen or not); wing length data were analysed using 3-way ANCOVA (main effects: sex, substrate and whether chosen or not, sugar concentration = covariate). Finally, the association of aggregation and survival or wing lengths in protocol b) were tested using SRC.

Results

Effects of substrate and sugar on egg numbers and distributions

As in previous experiments, females laid more eggs in total on yeast-dipped grapes than plain (MWU test on total eggs; $Z = -6.85$, $p < 0.001$, $N = 281$, mean eggs on dipped = 26.88; SD = 14.09; mean on plain = 15.42, SD = 10.66). The degree to which eggs were aggregated also differed between substrates with eggs on dipped grapes being significantly more aggregated (median $I = 2.25$, IQR = 2.01) than on plain grapes

Table 7.1. Mean survivorship (proportion) per grape half (calculated as adults emerging per number of eggs) and mean male and female wing lengths for the two substrate types, as well as results of MWU tests for comparisons between substrates.

variable	substrate		MWU			
	plain mean (SD)	dipped mean (SD)	Z	p	N plain	N dipped
survival	0.19 (0.36)	0.49 (0.31)	-7.44	< 0.001	281	876
♂ wing	1.31 (0.06)	1.31 (0.05)	- 0.69	ns	142	413
♀ wing	1.47 (0.08)	1.49 (0.09)	-0.34	ns	139	463

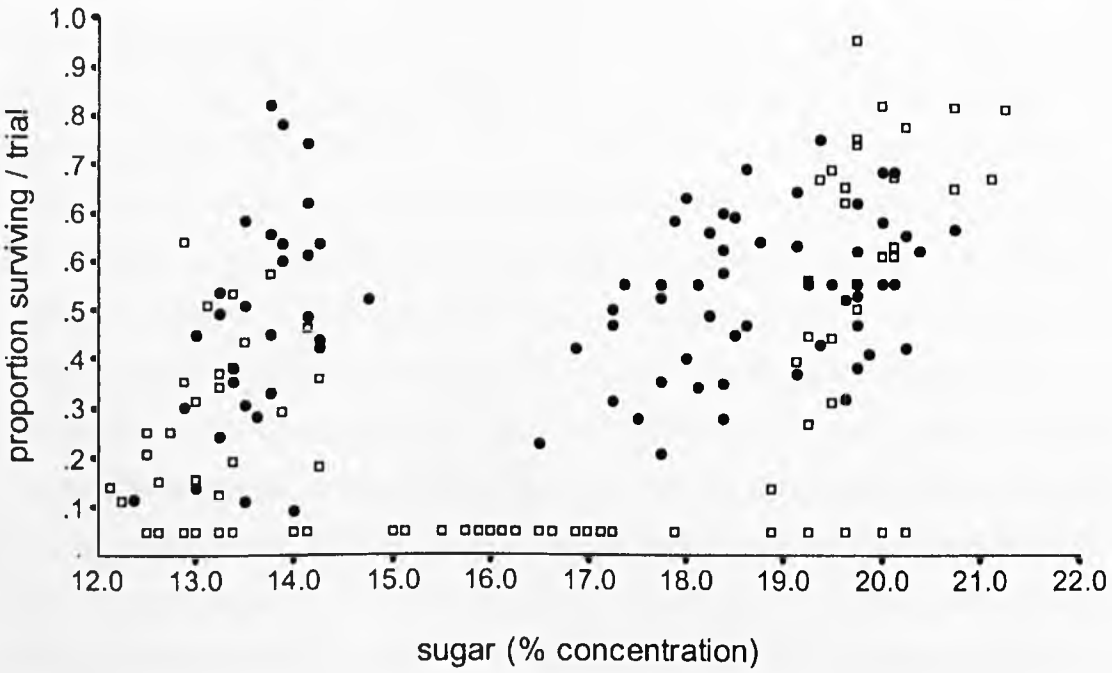

Fig. 7.3. Mean proportion surviving (calculated as adults emerging per number of eggs) during trials with varying mean sugar concentrations for four grape halves. Open squares = plain grapes; solid circles = dipped grapes.

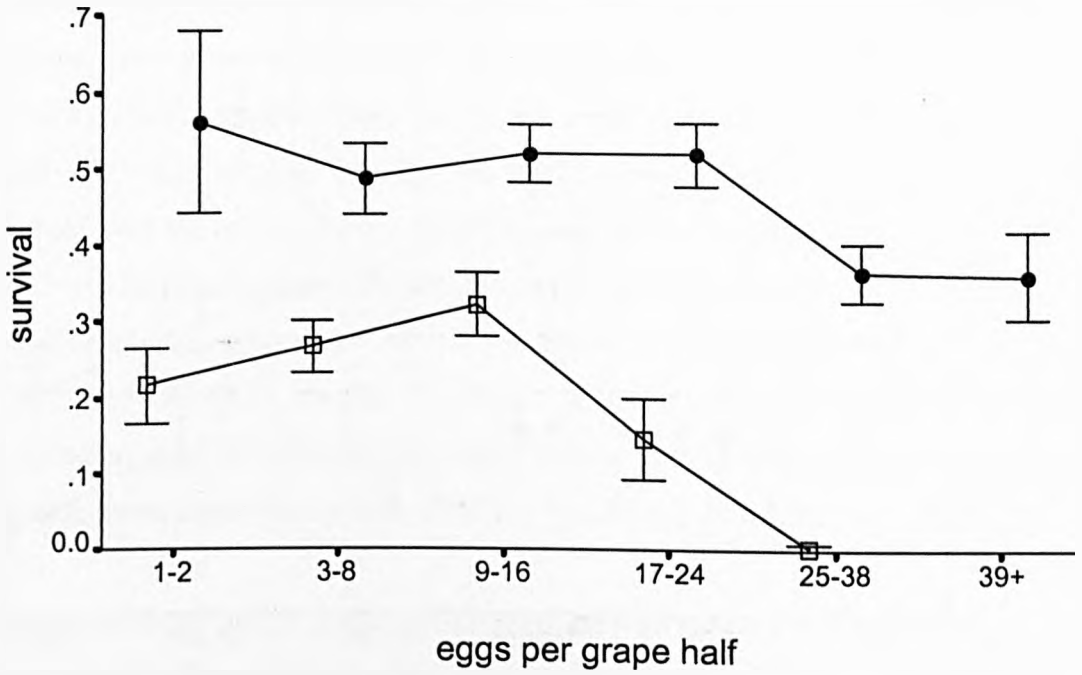

Fig. 7.4. Survivorship (mean proportion ± 1s.e.) in response to different initial egg densities on grape halves, subdivided into classes. Open squares = plain grapes; solid circles = dipped grapes.

Table 7.2. Results of ANCOVA on wing lengths of *D. simulans* emerging from grapes in response to substrate (plain, dipped), sex (male, female), density (class 1-6) with sugar concentration as the covariate.

Source	**SS**	**d.f.**	**F**	**p**
sugar (covariate)	0.015	1	0.42	ns
medium (M)	0.026	1	0.74	ns
sex (S)	0.808	1	23.25	< 0.001
density (D)	0.098	5	2.87	0.018
M x S	0.006	1	0.17	ns
M x D	0.106	5	3.05	0.005
S x D	0.016	5	0.46	ns
M x S x D	0.018	5	0.52	ns
error	19.771	569		

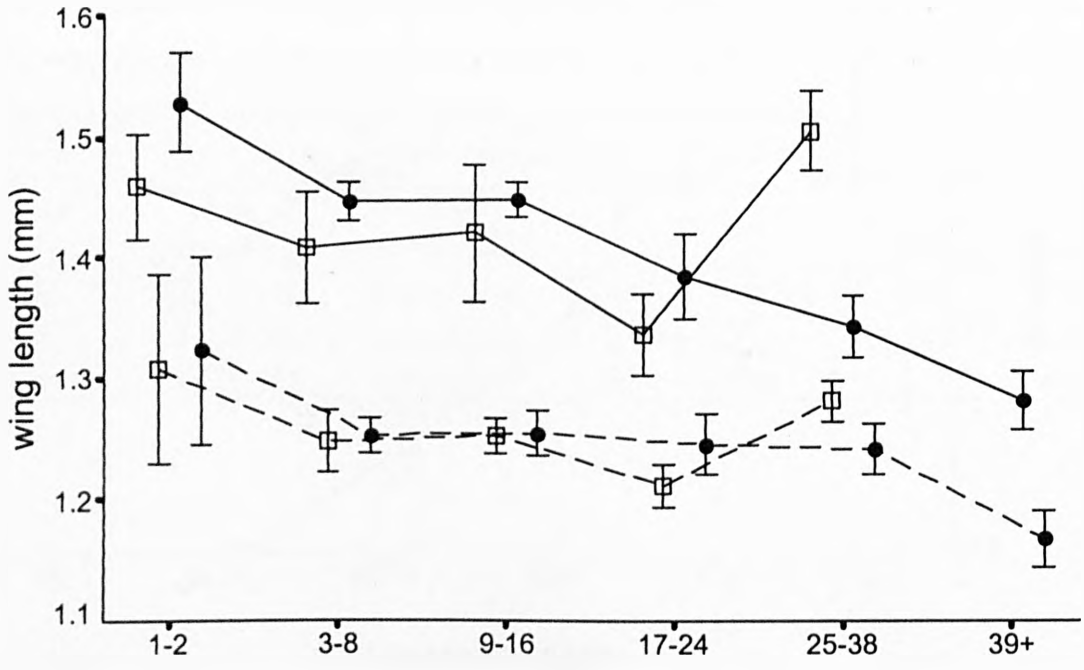

Fig. 7.5. Wing length (mean ± 95% C.I.) for male and female *D. simulans* in response to initial egg density in grape halves. Open squares = plain grapes; solid circles = dipped grapes; solid lines = ♀; broken lines = ♂.

Table 7.3. Results of ANCOVA on survival (proportion adults emerging out of 10 transferred larvae) in response to substrate (plain, dipped), whether a grape had been chosen for oviposition (yes, no) and grape sugar concentration (covariate).

Source	**SS**	**d.f.**	**F**	**p**
sugar (covariate)	5.957	1	143.03	< 0.001
medium (M)	3.864	1	92.44	< 0.001
chosen (C)	0.098	1	2.37	ns
M x C	0.220	1	5.34	0.022
error	6.414	154		

(median I = 1.15, IQR = 1.98; MWU test on I; Z = -5.78, $p < 0.001$; N = 281). Sugar concentration in grapes affected egg numbers only on dipped grapes; females laid more eggs during trials with lower mean percentage sugar in grapes (SRC; r_S = - 0.67; $p < 0.001$, N = 139; see Fig. 7.1). Similar results were obtained if egg numbers on each grape half were related to the corresponding sugar concentration (SRC; r_S = -0.15, p = 0.007, N = 556 but note that each female contributes four observations). The association on plain grapes by contrast, was positive although non-signficant (SRC r_S = 0.21, p = 0.157, N = 139, see Fig. 7.1). Egg distributions (I) were not affected by sugar concentration on plain grapes but on dipped grapes, eggs became less aggregated with increasing mean sugar during trials (SRC; r_S = -0.25, p = 0.023, N = 139; Fig. 7.2).

Effects of substrate, sugar, density and aggregation on survival and body size

a) Survival was affected by substrate type. Significantly more adults emerged from yeast-dipped grapes. Male and female wing lengths were not affected by substrate (Table 7.1). In investigating the effect of sugar concentration on survival, it was impossible to disentangle any effect from that of density. SRC tests for both substrate types showed that both on plain and dipped grapes, survival increased with increasing sugar concentration (SRC; r_S = 0.43, $p < 0.001$, N = 263 on plain grapes; and r_S = 0.18, p = 0.005, N = 233 on dipped grapes; see Fig 7.3). On plain grapes however, survival decreased with increasing initial egg density, although the resulting p value was only just below the 5% significance level (KW test; χ^2 = 9.82, d.f. = 4, p = 0.044; see Fig. 7.4). Egg density had no effect on dipped grapes (KW test; χ^2 = 8.75, d.f. = 5, ns, see also Fig. 7.4). The relative influences of density and sugar on wing length were more easily separated. Sugar concentration had no effect on the size of the emerging progeny, neither did the type of substrate used (Table 7.2). Sex obviously affected wing lengths with males being significantly smaller than females (Fig. 7.5). There was clear density-dependence but the response depended on the substrate which explains the significant interaction term (Table 7.2) for medium and density. While on dipped grapes adults emerging from the highest density class were smaller than at lower densities (Fig. 7.5), this was not observed on plain grapes, where, if anything, adults of either sex increased in size.

The way females distributed their eggs had little effect on survival or size. For both grape treatments, there was no significant relationship between the index of aggregation,

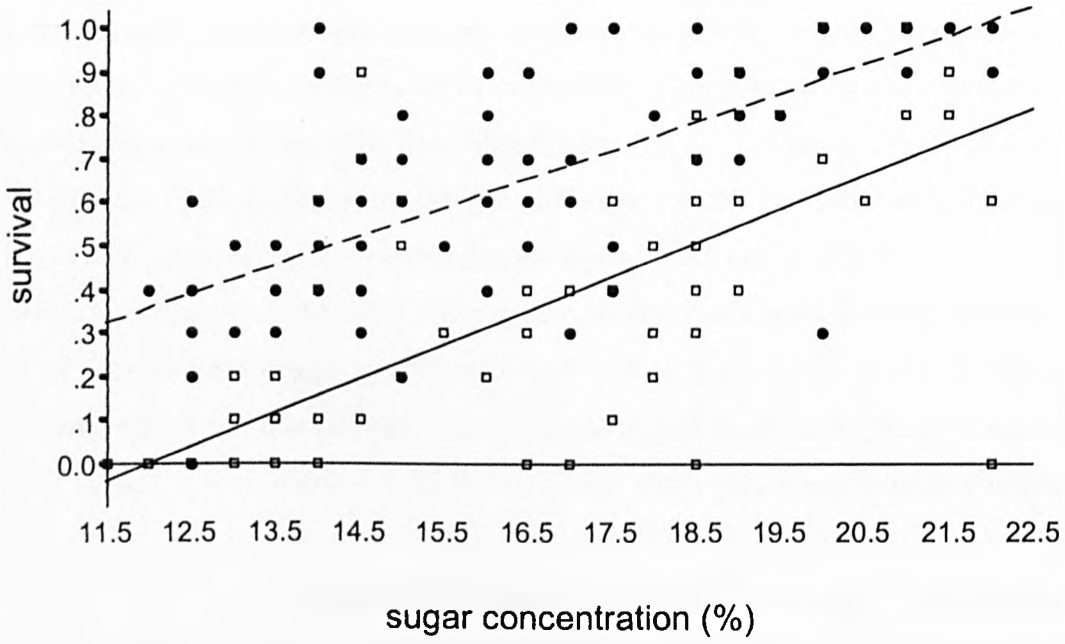

Fig. 7.6. Proportion surviving per grape half (out of 10 transferred larvae) in response to grape sugar concentration. Open squares = plain grape; solid circles = dipped grapes; solid line = regression line on plain grapes ($r^2 = 0.47$) ; broken line = regression line on dipped grapes ($r^2 = 0.50$).

Table 7.4. Results of ANCOVA on wing length in response to substrate (plain, dipped), whether a grape had been chosen for oviposition (yes, no), sex (male, female) and grape sugar concentration (covariate).

Source	**SS**	**d.f.**	**F**	**p**
sugar (covariate)	0.055	1	4.97	0.020
medium (M)	0.077	1	16.48	< 0.001
chosen (C)	0.016	1	3.50	0.064
sex (S)	0.443	1	95.42	< 0.001
M x C	0.022	1	4.77	0.031
M x S	0.011	1	2.34	ns
C x S	0.021	1	4.60	0.034
M x C x S	0.003	1	0.27	ns
error	1.476	135		

I and either the proportion of eggs generating adults or their wing lengths (non-significant SRC tests).

b) Results for protocol b) confirmed that survival was enhanced with increasing sugar concentration in grapes on both substrate types (Table 7.3, Fig. 7.6). Survival again was higher on dipped than plain grapes. In addition, it was possible to investigate whether the original preferences of females for oviposition had any effect on the survival of the ten transferred larvae. Table 7.3 shows that although overall the effect was non-significant, the significant interaction term indicates that female choice had some effect on one of the two substrates (Table 7.3). The mean values suggest that on plain grapes, survival was higher on grapes that had been chosen by females (mean survival = 0.43(SD = 0.32) on chosen grape halves compared to 0.26 (SD = 0.30) on those that had contained no eggs) while such differentiation was not observed on dipped grapes (0.63 (SD = 0.25) compared to 0.69 (SD = 0.25) respectively). The analysis of wing length data showed that both sex and substrate had highly significant effects on size (Table 7.4, see also Fig. 7.7a and b). The effect of whether or not a grape had been chosen for oviposition by a female on wing length was just non-significant but again, the significant interaction term for substrate and whether or not a grape halve had been chosen indicates that on one substrate, both male and female sizes were influenced; on plain grapes flies emerging from grapes that had been chosen were slightly larger but the effect was more pronounced for males (mean = 1.29 (SD = 0.06) on chosen compared to 1.17 (SD = 0.08) for males and 1.48 (SD 0.10) compared to 1.44 (SD 0.11) for females respectively). The original index of aggregation, *I*, had no effect on survival or wing lengths of the transferred individuals in a trial.

Discussion

Like other insects, *Drosophila* are capable of detecting the presence and assessing some qualitative aspects of resources by two separate mechanisms. They can detect chemical stimuli through sense organs in the antenna (Ashburner 1989) and they can perceive the 'taste' of a resource via chemoreceptors in the forelegs (taste hairs in the tarsi; e.g. Cadieu 1989) and in the mouthpart (taste hairs in the labellum; e.g. Schnuch & Seebauer 1998). Sensing through the antenna is probably involved in detecting resource chemicals over a distance while a taste response is only possible when flies are in

contact with the substrate. *Drosophila melanogaster*, for example, orientate towards

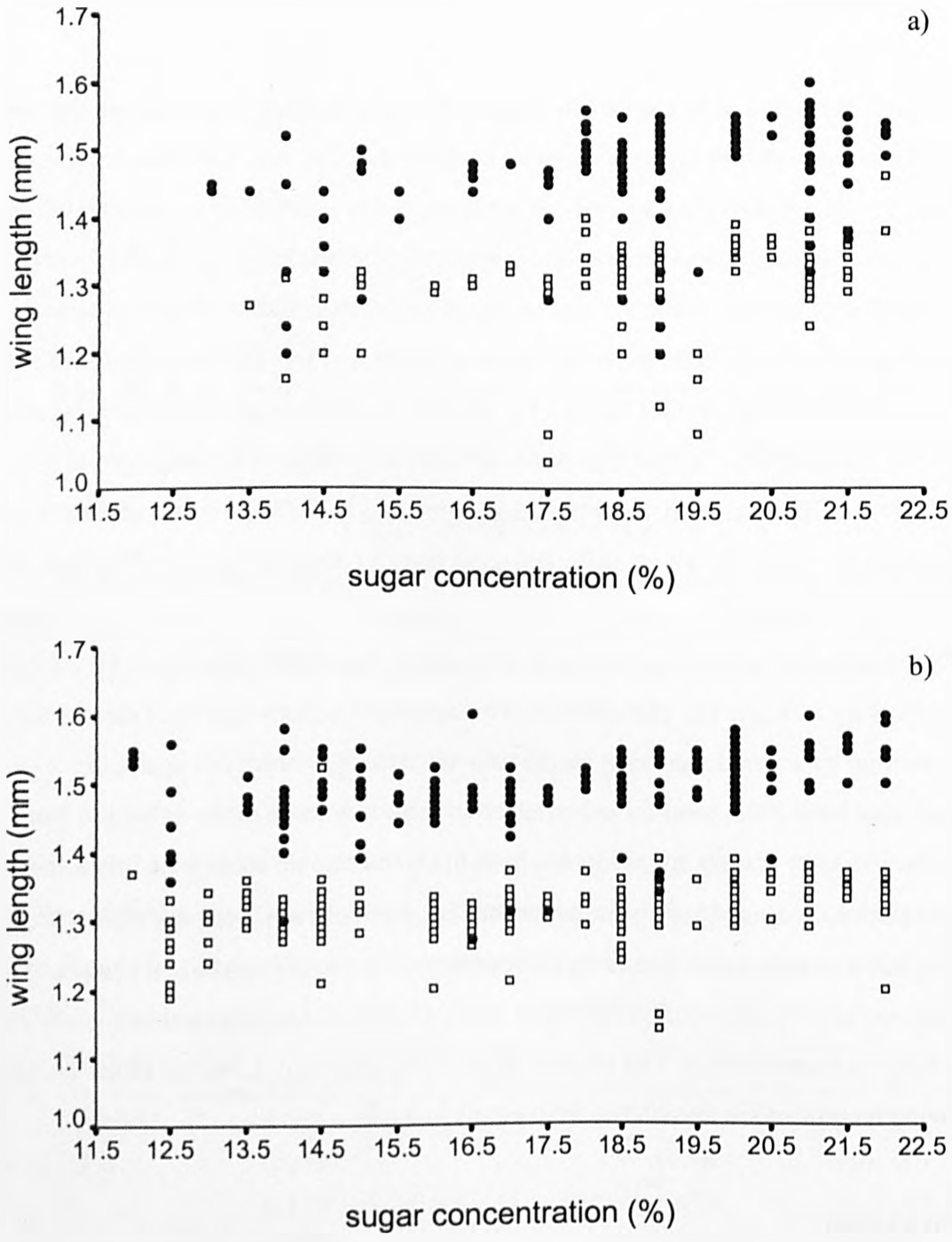

Fig. 7.7. Wing lengths in response to grape sugar concentration for plain (a) and dipped (b) grapes. Open squares = males; solid circles = females.

fermenting fruit at about 40 cm but when the antennal response was hindered (e.g. by painting over antennae), flies responded only when very close to the fruit (see Shorrocks 1972). There is no doubt that insects can assess the sugar content of resources. Nectivorous lepidopteran species can discern both the type of sugar present and its concentration (e.g. Erhardt 1992; Wei *et al.* 1998) and *D. melanogaster* feeding behaviour is influenced by sucrose concentration, depending on the nutritional state of the adults (Edgecomb *et al.* 1994). In other insects the chemoreception upon contact with the substrate influences host selection and oviposition behaviour, as demonstrated, for example, in the cabbage root fly, *Delia radicum* (Roessingh *et al.* 1997) and in whiteflies, *Bemisia argentifolii* (Bentz *et al.* 1995). Similarly, Mitchel & Soucie (1993) showed that in blowflies (*Sarcophaga bullata*) taste was more important in determining the larviposition behaviour of this species than olfaction.

Grape sugar content is determined by the length of time fruits are left to ripen before harvesting. Once harvested, fruits do not continue to ripen but there may be substantial differences between fruits even on the same bunch depending on pre-harvest exposure to sunlight (Peynaud & Ribereau-Gayon 1987). This study confirmed that sucrose concentration in commercially available grapes, all of the same variety, can vary by as much 11.5%. The main variation was between different bunches but likewise, grapes of one bunch were highly variable, and within one grape the half containing the peduncle was often sweeter. Female *D. simulans* in this study responded to varying sugar concentration in grapes only when yeast was present, i.e. on dipped grapes. Although increasing sugar concentration significantly increased fitness on both substrates, the response on dipped grapes was to lay fewer eggs that were more scattered, the more sugar was present in the resource. On plain grapes, the reverse tendency was observed but the response was non-significant. This may be explained by a number of different observations. Fruit flies respond to a variety of organic chemicals that are found naturally in fermenting fruits, including amyl and ethyl alcohol, acetic or lactic acid and ethyl acetate (Shorrocks 1972). These compounds are the metabolic products of fermentation by yeasts (e.g. Pfaff & Starmer 1987) and it is likely that their concentration and thus the intensity of the cue vary with the amount of sugar available for yeast assimilation. The intolerance of *D. simulans* to high ethanol concentrations is well documented (e.g. Parsons & Spence 1981) and this may explain the negative correlation of egg numbers and sugar concentration. On plain grapes by contrast, the presence of fermenting micro-organisms is likely to be highly variable (see also Chapter

3) which is why there is no significant response to sugar. In fact, it may be that the non-significant positive response to sugar is explainable because *D. simulans*, although not able to detect sugar before arriving at a patch, respond positively towards it once they can taste it (see also Mayor *et al.* 1987). Detection of sugar on yeasted grapes may be thus pre-arrival, mediated through yeast metabolic activity, but post-arrival if little yeast is present.

Sugar availability clearly increased survival and, once density-dependent effects of larval competition were removed from the analysis, also wing lengths. As discussed in Chapter 3, although larvae can survive on yeast-cells alone, they perform much better if other nutrients are supplied (e.g. Sang 1949; Kearney & Shorrocks 1981) and competition for nutrients (and hence density-dependent effects) are likely to be reduced on substrates with more sugar. There are other mechanisms by which sugar may improve survivorship and fitness. Bruins *et al.* (1991) showed that while sensitivity to light (especially in the absence of yeast) in *D. melanogaster* larvae can markedly increase mortality and delay development, sucrose supplement to the substrate offered protection from such sensitivity. Additionally, Pecsenye *et al.* (1996) demonstrated that sucrose content of the medium affected ethanol tolerance in *D. melanogaster*, with larvae at high sucrose concentrations being more tolerant to ethanol stress (see also Tarin *et al.* 1991). There is evidence however, that high sucrose concentrations can also have negative effects in *Drosophila*; Wang & Clark (1995) found that a diet medium containing 10% w/v sucrose could reduce adult live weight, total protein and enzyme activity in *D. melanogaster*.

Results for plain grapes suggest that sugar is not the only factor influencing survival or adult size. Here, grapes that had been chosen by females for oviposition in the basic trials, later generated a higher proportion surviving and larger adult sizes in ten transferred individuals. Since no such effect was observed on dipped grapes, the most likely explanation is that the reason why they were chosen and the improved fitness is due to the presence of beneficial yeasts. The absolute requirement of *Drosophila* for yeasts has been stressed before (see Chapter 3; Kearney & Shorrocks 1981). On non-yeasted grapes, the distribution of yeasts is likely to be probabilistic (see Chapter 3) and represents a factor for qualitative differences between patches not present on dipped grapes. Since the top layer of the grape halve was sliced off before adding larvae, it is unlikely that yeasts transferred by the females themselves (e.g. Begon 1982) played a

large part in subsequent performance of larvae. This is also supported by the observation that on plain grapes, transferred larvae (protocol b) performed generally better than those that hatched naturally from eggs (protocol a). The main reasons for this are that mortality in protocol a) is likely to be an overestimate due to a proportion of non-viable eggs but also that transferred larvae had the benefit of having started off with an unlimited supply of dead yeast cells on the collection plates, some of which were also transferred onto the grape halves.

The results however, have other very interesting implications. Effectively, I have recorded the oviposition choice of individual females over 24 hours on four possible patches and followed the implications of that choice for the survival and subsequent fitness of their offspring. Results supported clearly what the combined findings of the study in Chapter 3 and 6 already suggested: the number and distribution of eggs by individual females clearly reflect qualitative differences of the substrate. If the quality of substrates is poor (plain grapes), fewer eggs are laid and they are more scattered probably as a result of the increased likelihood of females to leave a poor quality patch to search for another. The egg numbers and distributions (clutch sizes) of females are therefore highly variable. Although oviposition behaviour is clearly adjusted according to substrate type (and to some extent to sugar concentration), there are still density-dependent effects suggesting that oviposition choices probably have to be viewed in the context of an optimal foraging strategy (e.g. Skinner 1985; Mangel 1987). This will be discussed in more detail in Chapter 8.

Chapter 8 - General conclusion

This work has clearly demonstrated that although looking for the adaptive significance of ecological processes can be fruitful and can lead to interesting revelations, it is difficult to address the ultimate causes of such processes before the mechanistic or proximate explanations have been found. Initially, the aim of this work was to look for direct evolutionary explanations, for selective forces that could account for the propensity to aggregate whose prevalence in nature seemed to be contradicted by theoretical models and our understanding of the process. It is extremely difficult however, to prove that a trait, such as aggregation, evolved because of a particular function (e.g. Clutton Brock & Harvey 1984; Futuyma 1986). This difficulty became immediately obvious when in Chapter 2, I showed that although birds, at least, had the potential to act as a selective force by avoiding insects and possibly aggregates of insects in shared resources, to prove such a link, many more questions would need to be answered. A common approach is to ask comparative questions, for example, how often has aggregation arisen independently during phylogeny and did these evolutionary events transpire in the same selective context, i.e. bird predation? Although aggregation is generally assumed to be a widespread phenomenon (e.g. Shorrocks & Rosewell 1986), most studies have examined its occurrence in fruit- or fungi-breeding Diptera, with *Drosophila* being often the only genus where classification to species has been attempted (Rosewell *et al.* 1990, Sevenster 1996), in a number of carrion-breeding fly species (Ives 1988a; 1991; Kouki & Hanski 1995) and in dung beetles (reviewed by Hanski 1990). The consensus is that for insects using patchy and ephemeral resources (and probably for many that do not), aggregated distributions are the norm rather than the exception. If there is differentiation between species and patterns across taxa, such records are not available and generally very little is known about the specific ecology of particular insects. The incidence of vertebrate aversion to insect-infested resources addressed in the literature review certainly lacked general patterns and even the extent of resource use overlap and hence potential predation rates were variable and depended on the local species compositions. To answer questions like whether the relative predation rates differ for insects that utilise shared resources but vary in the degree to which they aggregate, or similar generalised approaches, was not possible. Aggregation may hence

sometimes confer advantages by protecting against vertebrate predators but it remains questionable whether the widespread occurrence of aggregation in insects that overlap in many but not in this particular aspect of their ecology does not also warrant further, perhaps more general explanation.

Vertebrates are, of course, not the only predators of aggregating insect species. Ants and rove beetles, for example, have been shown to reduce the survivorship of drosophilid larvae, sometimes by as much as 90% (Escalante & Benado 1990; Ståhls *et al.* 1989; Lewis & Worthen 1992). Worthen (1989) demonstrated that predation on adult, mycophagous *Drosophila* by rove beetles could mediate interspecific competition in such a community. This effect was not confirmed for ant predators but mortality through predation was still increased in some cases by as much as 60% (Worthen *et al.* 1993; see also Worthen *et al.* 1994). While these studies emphasise that coexistence in *Drosophila* communities can be mediated through processes other than aggregation, it is also likely that such predators have an impact on the density and distribution patterns of their prey. The effect of prey abundance on predator distributions represents an extensive area of research especially when host-parasitoid relationships are included. (e.g. Hassel & May 1973; Chesson & Murdoch 1986; Wade & Murdoch 1988). Predator response types to prey density (see Holling 1959), models like the ideal free distribution (e.g. Fretwell & Lucas 1970) and aggregative responses to patchy distributions of prey (e.g. Hassel & May 1974) are well known approaches, and certain models predict that prey species at low densities or in low density patches can be more affected by predation or parasitism than those at high densities (e.g. Morrison & Strong 1981; Hassel 1982). In cases where such a response is prevalent, coexistence through predation could be effected more indirectly than in many of the conventional models (e.g. Holt 1977; Jeffries & Lawton 1984), because such predatory responses confer advantages to organisms that aggregate. There is evidence that for parasitoids at least, both types of density-dependent effects, i.e. refuges for individuals of a prey or host species at either high or low densities, are common (Lessells 1985; Stiling 1987). Jaenike & James (1991) showed however, that rates of infection by the nematode *Howardula aoronymphium* in several species of *Drosophila* were density-independent. This is contrasted by a study of mycophagous *Drosophila* where density-dependent parasitism was demonstrated although there was no evidence of inverse density-dependence (Driessen & Hemerik 1991). In the absence of many more such studies (even fewer are

available for predatory species of aggregating insects) it is impossible to look for general patterns but even this limited number of studies suggest that predation or parasitism are unlikely to operate as driving factors for aggregation over a wider taxonomic range. They may represent an explanation in specific cases but are unlikely to provide a general mechanism.

Allee effects seem more likely explanatory factors since they are intrinsic to the dynamics of populations, especially when not dependent on interspecific interactions or environmental factors, which are almost certainly variable across the wide taxonomic span of aggregating insects. Allee effects were observed in *D. simulans* (Chapter 3), but they were generally weak and very dependent on the precise characteristics of the breeding sites. There was evidence that competitive interactions between insects and colonising yeasts could produce Allee effects. Although this represents a novel mechanism for Allee effects in *Drosophila*, its generality in the context of aggregating insects is questionable and the frequency with which such conditions are encountered in the field remains unknown. Even in the narrow system studied here, the effect was weak and specific; for other systems different mechanisms that cause Allee effects would have to be found. From the results it is not possible to exclude Allee effects as factors in aggregation but they are unlikely to play an important role, especially when population densities of competing species are high. As far as the frequency and impact of Allee effects are concerned, theory is currently in advance of data, especially from field studies (e.g. Stephan & Wissel 1994; Cushing 1994; McCarthy 1997; Amarasekare 1998; Lande 1998). Some exceptions include Allee effects demonstrated in natural populations of butterflies (Kuussaari *et al.* 1998) and of plants (e.g. Lamont *et al.* 1993; Fischer & Matthies 1998; Groom 1998). These populations are commonly small and/or of rare, endangered species and the effect is mediated through aspects of sexual reproduction.

The second part of the study emphasised the importance of understanding the processes that lead to aggregation in a mechanistic rather than an evolutionary sense, as it became obvious that these were still poorly understood. In Chapters 4 to 7, I presented several investigations into the factors that influence oviposition decisions of individuals. Here, I

Table 8.1. Typical numbers of Diptera (immatures or emerging adults) found in field samples of fruit and fungi.

Species	resource type	sampling type	density (number per resource unit)		reference
			range	mean (range)	
various *Drosophila*	neotropical fruits (field sample)	emerging adults (rearing data)		7.98 (year 1) 20.98 (year 2)	Sevenster & Van Alphen (1996)
		larvae (dissection data)		28.38	
7 species of *Drosophila*	fruits and vegetables from fruit markets	emerging adults (rearing data)	0 - 45 depending on resource type	22.6	Atkinson & Shorrocks (1977)
various Diptera	Australian fruits (field sample)	emerging adults (rearing data)		0.11 - 33.07 (mean = 5.40)	Atkinson (1985)
D. melanogaster	fruit	emerging adults	1 - 18	3.87	Rosewell *et al.* (1990)
D. funebris	fungi		1 - 10	1.10	
various *Drosophila*	12 types of Australian fruits	emerging adults (rearing data)		0.29 - 12.25 (mean = 2.5)	Atkinson & Shorrocks 1984
D. subobscura	ornamental plum	eggs (dissection data)	1 - 39	3.55	
various *Drosophila*	fungi	emerging adults	0 - 90		Shorrocks & Charlesworth (1980)

found that individual oviposition behaviour in *D. simulans* was sufficient to generate aggregated egg distributions. An important observation was that egg distributions on four resource patches were the products of different clutch sizes, i.e. eggs were laid in clusters before females left patches to move onto the next (see Chapter 5). The size of clusters depended on many factors: light availability, the ease with which resources could be detected, accessed or left again, the size of the available oviposition surface and, importantly, on qualitative aspects of the resource. With so many factors influencing clutch size, the negative response to selection in Chapter 5 was explicable. Results of Chapter 6 suggested that aggregation which is actually caused by attraction of females to each other, i.e. gregariousness (see Chapter 5), was weak. At low densities, females showed less overlap in patch use than could be expected if associations were random, at higher densities distributions became less aggregated.

There are several lines of evidence that suggest that in the field aggregation may also be largely due to clustered egg-laying rather than to aggregation of ovipositing females. Jaenike & Selander (1979) showed, using electrophoretic evidence, that *Drosophila* species emerging from single fungi collected in the field were often the offspring of one or only a few females, suggesting that some *Drosophila* do cluster eggs in the wild. The published record for egg numbers per breeding site is not extensive as most studies do not give records for individual sites. The number of emerging adults, published in some studies, is, of course, likely to be an underestimate of actual egg numbers due to pre-adult mortality, especially due to competition. Sevenster & Van Alphen (1996) addressed this problem and demonstrated that immature *Drosophila* in neotropical fruits had an average survival rate of about 0.7. How general this is remains unknown, especially in temperate regions. Table 8.1. summarises some studies where numbers per breeding site of either emerging adults or eggs could be extracted; sometimes only mean ranges are given and because of the aggregated nature of egg-laying means are a poor description of the data. The numbers sampled were generally low, with some exceptions, and if this is related to the very clear results of Chapters 5 and 6 it seems more likely that numbers represent the reproductive effort of one or a small number of females rather than very small clutches of many. The oviposition responses of *D. simulans* demonstrated in this study (and of *D. melanogaster* and *D. subobscura*, pers. obs.) discredit the idea of random arrival at a site or random probability of leaving after laying an egg which would generate the latter pattern. The

aggregation measured in studies like the ones listed in Table 8.1, therefore could in the main be due to the distribution of clutches of different sizes by different females. Atkinson (1979) suggested that large-bodied *Drosophila* species laid large clutches of small eggs whereas the opposite was true for small-bodied species. Courtney *et al.* (1990) reported that in the mycophagous *Drosophila suboccidentalis* the number of eggs a female laid before leaving a host depended on female egg load and breeding site characteristics, e.g. the species of fungi used.

The role of clutch sizes in aggregation theory has received considerable attention. Green (1986; 1988) argued that if aggregation was mainly due to clutch sizes, it would not stabilise coexistence in insect communities (but see Atkinson & Shorrocks 1988). Sevenster (1996), too, suggested that large clutches and density-dependent effects on clutch size behaviour would lead to erroneous estimates of coexistence mediated through aggregation. More recent theoretical work suggests however, that even if aggregation is solely due to large clutch sizes it can be sufficient to allow coexistence or, at least, to strongly stabilise and prolong coexistence time in communities (Heard & Remer 1997). For these predictions to hold true, certain assumptions about clutch sizes of species with different competitive abilities have to be made and these are not necessarily supported by the results of this study (Chapter 6). I would like to emphasise however, that before these issues can really be resolved direct measurements of clutch sizes for different, competing species in the field are required. The advance of molecular approaches and the more recent trend towards the application of genetic techniques to ecological problems has made this a far more feasible tasks.

While results generated in this study certainly add to the debate over clutch sizes, it is somewhat beyond the scope of the project to discuss these fully. It is important to note however, that if aggregation in the field is mainly due to clustered egg laying, the objectives of asking 'why?' change. During the last decade, there has been an abundance of theoretical papers concerning optimal clutch sizes in insects. From such studies it has emerged that ovipositing females probably adjust clutch size in response to changing costs of search for and travel among resource patches (Parker & Courtney 1984; Skinner 1985; Mangel 1987; Heard 1998). Females laying a few large clutches incur higher costs of sibling competition among their offspring than females that lay smaller clutches but this is

balanced by the cost of search and travel (and the chance of not finding another suitable patch). Heard (1998) demonstrated clutch-size adjustments in *Drosophila recens* and *D. subquinaria* in response to the frequency of suitable oviposition sites in the environment. Associations between travel costs and clutch size or patch selectivity have also been reported from other insects (Jackson 1966; Benson *et al.* 1975; Roitberg & Prokopy 1983; Courtney 1986; Messina 1991). My results from Chapter 4 confirmed that clutch sizes in *D. simulans* increased as resources became less accessible or detectable. Further, the significant link between the distribution of clutches and resource quality in terms of offspring survival and fitness and also the evidence for density-dependent effects (Chapters 3, 6 and 7), suggested strongly that underlying such behaviour is an optimisation strategy.

Although my results stress the importance of clutch laying for aggregation and there are no studies that unequivocally demonstrate that aggregation (of insects on patchy resources) in the field is not due to clutch size, this does not imply that aggregated distributions in the field are never caused by the congregation of ovipositing females. Even if females are trying to optimise their individual fitness by avoiding intra-specific competition (see Chapter 6), in reality females will be prevented from distributing their offspring evenly (even if all patches were of equal quality) because the movement between patches may be non-optimal (see Hanski 1990) and the distribution of adults (conspecifics and heterospecifics) when resources become available is likely to be highly governed by chance and by the dispersal abilities of different species (see also the notion of 'fugitive refuges' and 'priority effects', e.g. Shorrocks 1990). Increasing the density of ovipositing adults will increase patch use overlap, even if it increases intraspecific competition (see Chapter 6). In addition, other mechanisms are likely to operate. Results from Chapter 6 showed, for example, that patch use overlap between conspecific females could be enhanced by decreasing the number of good quality resources available. This indicates that there is a fine line between the separate processes that can generate aggregation and, more importantly, that generate coexistence. While the distinction is necessary and convenient when these processes are investigated, in nature it is misleading (see e.g. Shorrocks 1990). It should be stated here, that coexistence through spatial avoidance was never purported to be the only process leading to coexistence and the characteristically high species diversity of insects on patchy resources (Shorrocks 1990). Rather, in nature the separate mechanisms

are likely to act together to generate and maintain such diversity. The novelty of the aggregation model of coexistence was that it predicted coexistence in the absence of resource heterogeneity and resource partitioning (or other conventional mechanisms). It seems to me that in an evolutionary context, aggregation makes sense only if it is due to clutch-laying behaviour while the reasons why females may also congregate at oviposition sites are due to processes that are not novel. It may be that with our current understanding, we have to investigate more closely what the implication of clutch-laying are for coexistence and for this, the aggregation model of coexistence and all the theory it subsequently generated still provide a good theoretical framework.

Insects on patchy resources are, of course, not the only organisms that aggregate. Instead, individuals from most biological populations show distributions that are aggregated as opposed to random or uniform (Taylor *et al.* 1978), although the levels of aggregation exhibited probably differ, especially across a range of different population densities (see Hartley 1998; Gaston *et al.* 1998) and depending on scale. While the aggregation model of coexistence is limited to aggregating insects, it has become very clear that the spatial structure of populations and communities is an important concept which cannot be ignored in ecological research. Considering the spatial aggregation of species and their patterns has furthered our understanding of ecology in many ways but because of the generality of the pattern, there are likely to exist many mechanisms, both proximate and ultimate, that determine why different populations and species aggregate. Although the importance of spatial structure has been recognised and is supported by an overwhelming number of theoretical models, these determinants are still poorly understood. Methods of analysing and describing spatial dynamics of biological systems in more appropriate, and especially in mathematically explicit and deterministic ways are still in their early development as conventional approaches prove decreasingly appropriate. With the development of new tools and, importantly, the increasing number of empirical studies that explore spatial theory, we are sure to gain more exciting insights into ecological and evolutionary processes in the future that may, in some cases, challenge and revolutionise our current understanding.

Appendix 1 - *Drosophila* vial food

- in a 2000 ml flask mix:

Agar-agar	7.5 g
Sucrose	40.0 g
Yeast (*Saccharomyces cerevisiae*)	25.0 g
Solution X	12.5 ml
Solution Y	12.5 ml
Solution Z	75.0 ml
ddH_2O	400.0 ml

Solution X - 15.5 g $CaCl_2$ in 250 ml ddH_2O

Solution Y - 15.5 g $Fe^{2+}SO_4$ in 250 ml ddH_2O

Solution Z - 80 g potassium sodium tartrate
5 g sodium chloride
5 g manganous chloride
in 1500 ml ddH_2O

- autoclave at 15 psi for 15 min.
- after autoclaving add:

4 ml Nipagin solution - 10 g Nipagin (p-Hydroxybenxoic acid methyl ester) in 100ml ethanol

15 ml CBZ solution - 20 mg CBZ (Bavistin) in 100 ml ethanol

- pour mixture into vials when about 60°C; stopper with cotton wool; store at 4°C

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