

Aggregative egg distributions may promote species co-existence – but why do they exist?

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ABSTRACT

Although the effects of aggregated egg distributions for the co-existence of *Drosophila* species are fairly well understood, the driving forces that select for such egg distributions remain unclear. This study investigated the fitness consequences of *Drosophila* oviposition behaviour on natural breeding substrates under controlled conditions. We used fruits of *Sorbus aucuparia* as an oviposition substrate and recorded the clutch sizes that *Drosophila subobscura* females produce and the way in which individuals distribute their eggs across fruits. We found that *D. subobscura* females significantly aggregated their eggs. We tested the two most obvious hypotheses that might explain the distribution pattern of the eggs. Neither the hypothesis that larvae benefit from resource-dependent facilitation when feeding in groups, nor the hypothesis that *Sorbus* fruits might provide a negatively density-dependent refuge against parasitoid attack, is supported by our data. In contrast, our results suggest that flies should distribute their eggs regularly across fruits if the maximization of offspring survival is the selecting force. We discuss under which circumstances the observed distribution pattern might be adaptive.

Keywords: Allee effect, *Drosophila subobscura*, egg distribution, fitness, individual behaviour, parasitoids, refuge.

INTRODUCTION

Few ecologists dispute that a thorough understanding of patterns at the population or community level often requires the integration of mechanisms from lower levels, such as individual behaviour (Hassell and Southwood, 1978; Lomnicki, 1992; Sutherland, 1996). Interestingly, some well-studied phenomena lack such a foundation. For example, the aggregation model of co-existence claims to explain the high diversity in communities of insects that develop on patchy resources (Shorrocks *et al.*, 1979; Hanski, 1981; Ives, 1988; Sevenster, 1996). This model is based on the observation that larvae of a given species are often aggregated across resource patches that are ephemeral and unpredictable in space and time (del Solar and Palomino, 1966; Hanski, 1981; Atkinson and Shorrocks, 1984; Ives, 1988; Shorrocks, 1991; Sevenster and Van Alphen, 1996; Heard, 1998). Such aggregated

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egg distributions have an important effect that is of major interest to population and community ecologists: increased intraspecific competition relative to interspecific competition appears to allow the co-existence of competing species and to enhance species diversity. Whereas the co-existence model has repeatedly been supported by experimental evidence (Atkinson and Shorrocks, 1984; Atkinson, 1985; Shorrocks and Rosewell, 1987; Jaenike and James, 1991; Sevenster and Van Alphen, 1996), how and why such aggregated distributions of individuals occur is much less well understood. This is intriguing for several reasons. First, Atkinson and Shorrocks (1984) have suggested, and Burla *et al.* (1987) actually reported, that aggregated distributions in drosophilid flies arise from females distributing clutches of eggs randomly across patches, although Green (1986) has clearly demonstrated that egg aggregations that merely stem from flies laying clutches of eggs and distributing them in a random fashion across patches are insufficient to explain species co-existence. Second, other than some studies of mechanistic causes and a few theoretical considerations (e.g. Atkinson and Shorrocks, 1984; Shorrocks and Bingley, 1990), almost no studies have involved a functional approach – that is, investigations of the fitness consequences of oviposition decisions (but see Burla *et al.*, 1987, although their results are based on pseudo-replications). Thus, in spite of some speculation on this subject, the fundamental questions regarding the reasons that females produce clutches of several eggs and why they produce aggregations by ovipositing into resources that have previously been used for egg laying has remained unanswered for the 20 years during which the co-existence model of aggregation has existed.

One possible explanation for the aggregated distribution of eggs lies in the facilitation of gregariously feeding larvae (also known as the Allee effect; see Stephens *et al.*, 1999, for a recent definition). In *Drosophila*, such an effect has been reported from large natural (Courtney *et al.*, 1990) and artificial (Bakker, 1961) resources under experimental conditions. A possible explanation for this effect is that larvae in groups might be better at controlling microbial competitors than single larvae. However, as Courtney *et al.* (1990) noted, it is unclear whether this resource-dependent Allee effect generally occurs on all the types of resources that drosophilids use as breeding sites.

Additionally, or alternatively, refuges might be a driving force for the aggregation of eggs, given that larger groups of larvae are better at reaching refuges that exclude natural enemies such as parasitoids (i.e. a refuge-dependent Allee effect). Spatial refuges have recently attracted considerable interest because they readily explain insect oviposition decisions (Price, 1988; Romstöck-Völkl, 1990; Abrahamson and Weis, 1997) and have a stabilizing potential for host–parasitoid interactions (e.g. Hochberg and Holt, 1995; but see Murdoch *et al.*, 1996). Nevertheless, they have not yet been considered as an explanation for the aggregated egg distribution of drosophilid flies. This is remarkable for two reasons. First, spatial refuges have often been reported to lead to inverse density-dependent parasitism (Price, 1988; Romstöck-Völkl, 1990; Abrahamson and Weis, 1997), which, in turn, should select for aggregated egg distributions in the host insect. Second, parasitoids are a major source of mortality for drosophilid larvae (Janssen *et al.*, 1988; Driessen *et al.*, 1990). Although attack of hosts by drosophilid parasitoids is positively density-dependent on thin artificial media where host larvae cannot escape (Van Lenteren and Bakker, 1978; Van Alphen and Galis, 1983; Hertlein and Thorarinsson, 1987), interactions between hosts and parasitoids might be different on natural breeding substrates such as fruits. We have hypothesized that groups of larvae might reach spatial refuges faster than single larvae with respect to parasitoid attack.

Although local larval densities in the field might result from the actions of more than one ovipositing female, a complete lack of information on which individuals contribute to egg aggregations demands that we study the behaviour of individuals as a logical first step in our investigation. Such an approach is considered to be much more sensitive than the observation of groups of animals (see Thompson and Pellmyr, 1991) and provides information on inter-individual variability. We tested the clutch sizes that individual *Drosophila subobscura* females produce and the manner in which individuals distribute their eggs across *Sorbus aucuparia* fruits. Using fruits harbouring different egg densities, we analysed whether fly larvae benefit from a resource-dependent or refuge-dependent Allee effect on such small natural breeding substrates, and thus whether fly oviposition behaviour leads to optimal egg distributions.

MATERIALS AND METHODS

We used specimens from a local population of *Drosophila subobscura* Collin, which had been reared in a climatized chamber at $18 \pm 1^\circ\text{C}$, $70 \pm 5\%$ relative humidity and 16 h photophase on artificial medium, consisting of corn meal, saccharose, Agar, baker's yeast, the fungicide Nipagin and water, for approximately 10 generations. *D. subobscura* uses a variety of breeding substrates, including fruits, fungi and sap fluxes (Burla and Bächli, 1991). The small fruits of *Sorbus aucuparia* L. (rowan berries of diameter 9.5 ± 0.23 mm; mean \pm standard error) that we used in the experiments are supposed to be a major breeding substrate for this fly in summer and autumn (Begon, 1975; but see Burla *et al.*, 1987, for prevalence of flies in such fruits in Switzerland). Ripe uninfested rowan berries were picked from trees during the previous summer and allowed to decay for 14 days in a climatized chamber before being deep-frozen. For experimentation, the fruits were thawed and the skin of the fermenting fruits damaged with a scalpel to provide an oviposition site of approximately 5 mm diameter (19.6 mm^2 surface area) (note that rowan berries have to rot to become a suitable resource for *D. subobscura*; Shorrocks, 1982; Burla *et al.*, 1987). The flies were 10 days old, mated and, before the experiment, had been kept with an ample supply of fruits to avoid any perception of resource limitation that could affect clutch size decisions.

To study clutch size – that is, the number of eggs that a female lays on a fruit at a single visit – individual flies were presented with two rowan berries, placed 4 cm apart in a 10-cm long translucent plastic vial of 4.5 cm diameter and sealed with a foam stopper. The first fruit on which the fly laid eggs served as the experimental fruit on which the clutch size was checked immediately after the fly had terminated its fruit visit. The second fruit served as an alternative patch to provide a stimulus for the fly to leave the first fruit. Since fly oviposition activity follows a diurnal pattern and peaks around the 12th hour of the 16-h photoperiod, we initiated 35 replicates of the experiment in the 12th hour of the photophase. Each fly was only used once.

To obtain information about egg distributions of flies across several fruits, 122 replicates of an experiment were carried out in which individual flies were presented with eight rowan berries in a 4×2 grid with 4 cm between fruits in an experimental arena of $17 \times 12 \times 6$ cm. In response to the diurnal oviposition pattern of flies (see above), we transferred flies into the experimental arena 4 h into the photoperiod and removed them 24 h later. This ensured that flies were undisturbed in their activity period and that our experiment would capture the egg distribution of a single diurnal oviposition period. After removal of flies, we analysed the distribution of eggs across fruits. We hypothesized that, if larvae benefited

from resource-dependent or refuge-dependent facilitation, females would distribute their clutches such that egg distribution across 24 h should reflect optimal egg densities per fruit. To test whether variability in fruit quality among the eight fruits offered per female led to the distribution pattern found, we additionally conducted 25 replicates of experiments in which we offered eight Petri dishes of 10 mm diameter (approximately the same size as fruits, but an oviposition area of 66.5 mm²) with artificial medium containing 0.1 mg baker's yeast per patch per 24 h. In these experiments, differences in resource quality should definitely be absent. To test whether distribution patterns found under laboratory conditions matched the pattern in the field, we exposed 200–300 *S. aucuparia* fruits on different dates and at different sites under ornamental plants on the university campus. Fruits were placed on the soil such that fruit densities of approximately 100 fruits · m⁻² were reached. Such densities fall into the range of natural fruit densities (e.g. Shorrocks, 1982). A sub-sample of fruits was re-collected after 24 h, checked for eggs and reared until the emergence of adult flies.

As one measure of the fitness consequence of different egg densities, the numbers of flies successfully developing from single rowan berries were counted. Egg-infested fruits came from our egg distribution experiments in the laboratory and, to avoid pseudoreplicates, only one of the eight fruits per fly was incubated on moist granulated clay until adult eclosion.

In a further experiment, we presented rowan berries harbouring one of five different densities (1, 2, 4, 8, 16) of 2-day-old drosophilid larvae (i.e. the preferred stage; Van Alphen and Drijver, 1982) to the solitary parasitoid *Asobara tabida* (Nees) to determine whether a density-dependent refuge for larvae exists within such fruits. Females of a parasitoid strain from stock raised at the University of Leiden, the Netherlands were reared in 65-ml plastic tubes containing artificial medium with early instar *D. subobscura* larvae. Before the experiment, isolated females were given experience by allowing them to search for fly larvae on two rowan berries each containing five 2-day-old *D. subobscura* larvae. After 60 min, the fruits were removed; 10 min later, wasps were allowed to search individually for larvae on the experimental fruit and were observed continuously. We offered single fruits containing one of the above-mentioned fly densities. Because kairomones from the ovipositing female fly and from fly larvae might affect parasitoid searching behaviour (Galis and Van Alphen, 1981; Wiskerke *et al.*, 1993), care was taken not to add or remove larvae from experimental fruits. Thus, fruits with appropriate egg densities were chosen and egg hatch was controlled before using fruits in the experiments. In each experiment, the fruit had been placed on a layer of Agar in the centre of a Perspex ring (2 mm wide and 20 mm diameter) within Petri dishes of 4.5 cm diameter. Observations were terminated when a wasp left the area delimited by the Perspex ring. Each of 49 wasps was used only on a single experimental fruit. Successful parasitizations by *A. tabida* were verified by dissection of all larvae from experimental fruits. All experiments were conducted in the climatized chamber under the above-mentioned conditions.

RESULTS

Clutches produced by individual females during a single visit to a rowan berry comprised 3.6 ± 0.46 eggs (range 1–12 eggs, $n = 35$; Fig. 1) and 77% of clutches contained more than one egg. Since flies laid an average of 19.07 ± 1.06 eggs in one diurnal oviposition period, the resultant egg distribution across fruits could have varied from regular to aggregated, depending on how the clutches were distributed. Most of the flies significantly aggregated

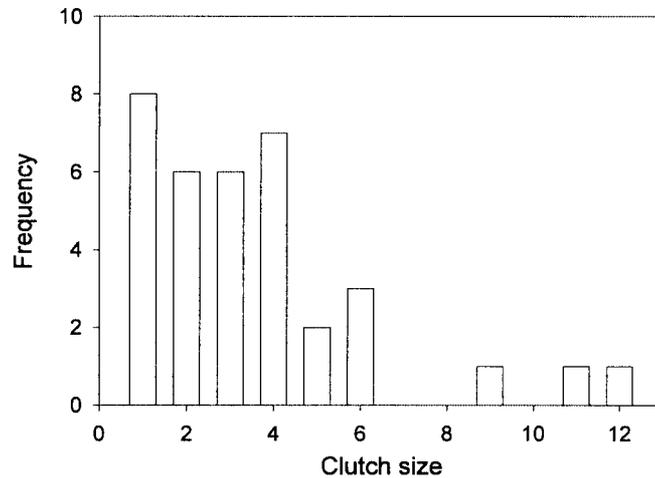


Fig. 1. Frequency distribution of clutch sizes laid by female *Drosophila subobscura* flies on a single visit to a *Sorbus aucuparia* fruit.

their eggs across the eight rowan berries (Fig. 2A, solid circles), and $43.4 \pm 2.3\%$ of the fruits remained uninfested. Not a single fly produced a significantly overdispersed egg distribution across the eight fruits, suggesting that clutches are randomly distributed or aggregated across fruits. The number of eggs per infested rowan berry was significantly higher than the average clutch size (4.23 ± 0.18 vs 3.6 ± 0.46 eggs; $P = 0.021$, Mann-Whitney U -test, $n = 157$), indicating that several fruits received multiple clutches. The amount of aggregation varied greatly between egg distributions of different flies, especially at low average egg densities. Moreover, the degree of aggregation showed a decreasing trend with increasing values for the mean number of eggs laid per fruit, approaching a Poisson distribution at extremely high egg densities (Fig. 2A). The same pattern of egg distribution was found in a simulation with compounding Poisson and logarithmic distributions of fruit visits and clutch sizes, respectively (Fig. 2B), and the confidence intervals of regressions for observed and simulated values broadly overlapped.

When flies were offered eight Petri dishes of 1 cm diameter (approximately the size of rowan berries) with artificial medium instead of fruits, flies left even more resource patches uninfested ($69.0 \pm 4.2\%$) and produced even stronger egg aggregations within one diurnal oviposition period (index of aggregation, J : for rowan berries 1.51 ± 0.15 ; for artificial diet 3.81 ± 0.4 ; $P < 0.001$, Mann-Whitney U -test, $n = 147$), whereas no difference was found in the average number of eggs that individual flies laid during the oviposition period (19.07 ± 1.06 vs 18.96 ± 1.61 ; $P = 0.804$, Mann-Whitney U -test, $n = 147$). This suggests that egg aggregation across fruits is not attributable to differences between fruits in their suitability as resources.

Furthermore, the pattern of egg distribution was similar in field-exposed fruits and laboratory experiments. Egg distributions found in samples of fruits that had been exposed in the field for 24 h had positive J -values throughout, indicating aggregation (Table 1). Five of seven samples produced a significantly aggregated egg distribution, whereas the remaining two distributions could not be separated from a Poisson distribution. The

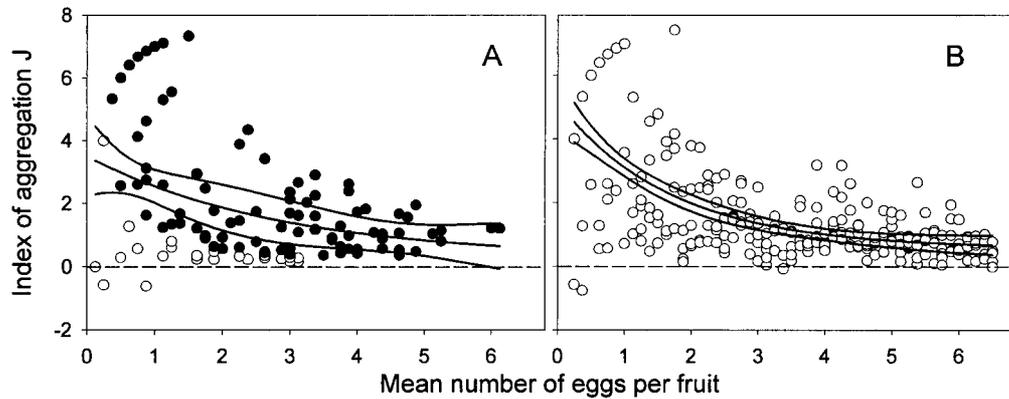


Fig. 2. (A) Egg distributions of individual *Drosophila subobscura* flies. Each data point represents the egg distribution of an individual fly across eight fruits of *Sorbus aucuparia* within one diurnal oviposition period (24 h). Egg distributions are expressed as the index of aggregation, J (see Sevenster, 1996, for an extensive discussion of this aggregation index), which gives the proportional increase in density relative to a Poisson distribution. Poisson distributions take a value of 0 (indicated by the dashed line), aggregated distributions take values >0 and regular distributions take values <0 . Distributions significantly different from a Poisson distribution [χ^2 tests of variance to mean ratio (Elliott, 1983), $P < 0.05$] are represented by solid circles; distributions that are not significantly different from a Poisson distribution are represented by open circles. By using an exponential equation of the type $y = y_0 + a \exp(-bx)$, which might, but does not necessarily, lead to an asymptotic approach towards a Poisson distribution with increasing egg densities, the best fit for the entire data set is obtained by the regression $y = -0.008 + 2.64 \exp(-0.262x)$, $r^2 = 0.106$, $P = 0.0012$, $n = 122$, which is given with 95% confidence intervals in the figure. (B) Simulated egg distributions across 8 fruits, assuming a compounding of Poisson and logarithmic distributions of visits to fruits and clutch sizes, respectively, as suggested by Atkinson and Shorrocks (1984). The clutch sizes were simulated with a mean clutch size of 3.6, as found in our clutch size experiments. Five replicates were simulated for each egg density between 0.25 and 6.5 eggs per fruit. The best fit to the data is obtained by the regression $y = -0.305 + 3.26 \exp(-0.242x)$, $r^2 = 0.272$, $P < 0.0001$, $n = 255$, which is given with 95% confidence intervals in the figure.

average number of eggs laid per egg-infested fruit in field samples was lower than the average clutch size that we had obtained in our clutch size experiments (1.34 ± 0.13 vs 3.6 ± 0.46 eggs per fruit; $P = 0.039$, Mann-Whitney U -test, $n = 42$). However, the J -values of field samples fell well within the range of J -values obtained in our egg distribution experiments with equivalent egg densities (Fig. 2A).

From both the results of our clutch size experiments and the egg distributions over one diurnal oviposition period, we expected to find some form of larval facilitation among larvae developing on *Sorbus* fruits. In contrast to our expectations, we did not find a resource-dependent Allee effect for the larvae developing in rowan berries. Rather, the highest survival rate was found for fruits with one egg, higher egg densities leading to severe mortality rates (Fig. 3). The best fit to the data was obtained with a regression model that is able to describe a wide range of forms of density dependence (Bellows, 1981). The model suggests a monotonically decreasing survival rate with increasing egg density (Fig. 3). Other functions, such as Gaussian or log-normal, which would be appropriate if an Allee effect

were present and if proportional survival were to have a maximum at some intermediate egg density, also gave a monotonically decreasing relationship (with the maximum of the function below one egg density of unity). They also had a poorer or similar fit compared with the regression model presented here. Despite the low survival at higher egg densities, a large proportion of eggs was laid such that fly offspring experienced crowding (Fig. 3).

Table 1. Egg distribution pattern of *D. subobscura* across field-exposed *S. aucuparia* fruits

	Sample						
	1	2	3	4	5	6	7
Fruits re-collected (<i>n</i>)	100	100	50	100	60	200	100
Percent infested	11	20	20	23	20	28	32
Eggs per fruit	0.15	0.22	0.28	0.34	0.42	0.44	0.6
Eggs per infested fruit	1.36	1.1	1.12	1.478	2.1	1.57	1.875
Index of aggregation, <i>J</i> *	5.22	0.24	1.55	1.56	2.65	1.92	2.06
χ^2 vs Poisson	5.84	0.67	2.52	6.45	19.24	20.38	25.43
d.f.	2	2	2	2	2	3	3
<i>P</i>	0.0539	0.7153	0.2837	0.0398	0.0001	0.0001	<0.0001
Power (1 - β)	0.77	0.77	0.63				
<i>a</i> compromise [‡]	0.12	0.12	0.24				

* See Fig. 2 for further explanation of *J*.

[‡] As suggested by power analyses with α/β - error ratio = 1 (Erdfelder *et al.*, 1996).

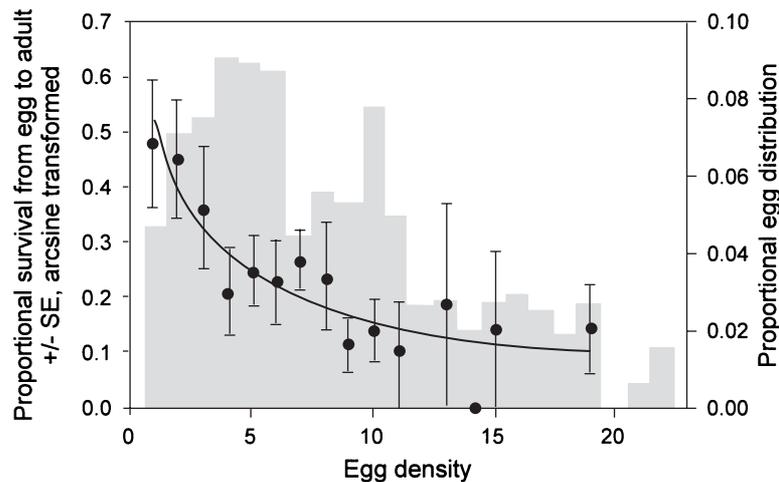


Fig. 3. The proportion of *Drosophila subobscura* flies surviving from egg to adult as a function of egg density in single *Sorbus aucuparia* fruits. We used regression of the type $y = (1 + (ax)^b)^{-1}$ (Maynard-Smith and Slatkin, 1973; see Bellows, 1981, for its suitability) to describe the effect of egg density on proportional survival: $y = (1 + (0.879x)^{0.765})^{-1}$, $r^2 = 0.806$, $P < 0.0001$, $n = 15$ (the original data set was reduced to data points that represent at least three replicates; however, inclusion of all data points does not change the pattern suggested by the function presented here). The distribution of eggs found in our egg distribution experiments is given by the shaded bars.

Moreover, our second hypothesis did not hold up either. Although rowan berries provide a refuge against parasitoids, with rates of parasitism levelling off below 40%, no negative density dependence and thus no refuge-dependent Allee effect was found (Fig. 4). Instead, parasitism was clearly positively density-dependent. The best fit to the data was obtained with a model suggesting an accelerating increase in density-dependent mortality at low larval densities up to approximately 5 larvae per fruit, and a decelerating increase of mortality for densities higher than 5 larvae per rowan berry (Fig. 4).

DISCUSSION

We have provided evidence for an aggregated egg distribution of drosophilid flies across natural substrates in field samples and in experiments in which individuals foraged in isolation under controlled conditions. Results from our clutch size experiments and the simulation of compounding Poisson and logarithmic distributions for fruit visits and clutch sizes suggest that the aggregated distributions found across fruits result from the production of clutches of several eggs per fruit visit. Thus, the aggregation of drosophilid eggs that is frequently found in the field is not necessarily the result of female aggregations on suitable oviposition sites, as might be deduced from the behavioural response towards so-called ‘aggregation pheromones’ (Hedlund *et al.*, 1996); nor can it be explained exclusively as a response to the limitation in oviposition sites or long travel times between sites (e.g. Heard, 1998). Our finding that the strength of aggregation decreases with increasing egg densities conforms well with a random distribution of egg clutches (Fig. 2B; Green, 1986). Although we cannot exclude that flies perceive differences in the quality between the fruits offered and adjust their egg distribution accordingly (for the effect of unsuitable sites on patterns of egg distribution, see Burla *et al.*, 1987; Shorrocks and Bingley, 1990), we found an even

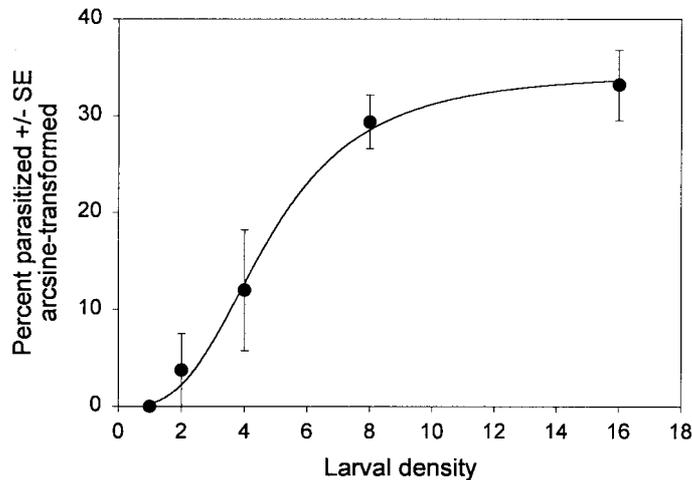


Fig. 4. The density-dependent proportion of parasitized *Drosophila subobscura* larvae in fruits of *Sorbus aucuparia*, when single females of the parasitoid *Asobara tabida* searched fruits harbouring 2-day-old fly larvae; the best fit to the data was obtained by the logistic regression $y = 34.42 (1 + (x/4.75)^{-3.05})^{-1}$, $r^2 = 0.996$, $P = 0.0039$, $n = 5$.

stronger aggregation of eggs when offering individual flies an artificial medium of identical quality in eight small Petri dishes. We draw the following conclusions from our results: First, flies appear to have perceived the quality of the artificial oviposition substrate to be better than the natural substrate and adjusted their egg distribution accordingly. Second, although it remains to be seen what kind of cue ovipositing flies use as a proxy for resource quality, both the experiments on clutch sizes and on egg distributions across rowan berries suggest that *D. subobscura* females perceive such fruits as a resource suitable for receiving more than a single egg. Thus, the large proportion of fruits that remain uninfested is not necessarily explained by the rejection of unsuitable fruits but by the deliberate aggregation of eggs across such fruits.

The intriguing question that follows from the observed clutch sizes, egg distribution pattern and high rate of fruits left uninfested is why females of *D. subobscura* aggregate their eggs. At least in our clutch size experiment, resources were not a limiting factor; indeed, the large number of uninfested fruits and the finding that the strongest aggregations were at low average egg densities in the egg distribution experiment suggest that fruits were not the limiting factor there either. Under such conditions, it would be expected that flies lay the clutch size that maximizes the fitness return per egg rather than producing clutches that maximize productivity per clutch – that is, the Lack clutch size (Godfray *et al.*, 1991). Neither of the two hypotheses that are the most likely candidates – a resource-dependent Allee effect (i.e. the facilitation of larvae feeding in groups) and a density-dependent spatial refuge against parasitoids – can explain the aggregation of fly eggs across rowan berries. In contrast, survival rates decreased monotonically with increasing larval densities per fruit, suggesting that female flies should lay a single egg per fruit. Moreover, rates of parasitism by *Asobara tabida* increased with increasing larval densities, again suggesting that female flies should lay a single egg per fruit. The density-dependent increase in parasitism follows a sigmoidal function and is thus in line with observations made on *A. tabida* with hosts on an artificial medium (Van Alphen and Galis, 1983). However, fruits obviously provide a refuge for *Drosophila* larvae, since the rates of parasitism are substantially below those reported on artificial media where larvae cannot escape parasitism (Van Alphen and Galis, 1983). Nevertheless, the refuge provided by rowan berries is most probably density-independent and thus cannot explain aggregative egg distributions.

Therefore, our results indicate that flies are unable to distribute their eggs optimally under the conditions tested. One possible explanation for the poor fit of the data with theory is that flies might be unable to measure resource quality correctly. Rowan berries are certainly not the only breeding substrate of *D. subobscura* flies, although they appear to constitute a major breeding source for such flies in summer and autumn (Begon, 1975). However, we have almost no information on the way in which flies measure resource value and derive an estimate of the number of offspring that might be able to develop on a given resource. The comparison of the egg distributions on rowan berries and artificial medium presented here and data from experiments with various yeast concentrations (M. Rohlf, unpublished) suggest that flies use mechanisms such as the available oviposition surface or the presence and concentration of yeast and sugars to estimate resource quality and to adjust their oviposition behaviour accordingly (i.e. oviposition behaviour appears to follow a stimulus-dependent reaction norm rather than a fixed pattern). Nevertheless, it is not obvious why flies should misjudge the carrying capacity of an important resource, and not only aggregate their eggs when confined to a limited amount of resource items (the egg distribution

experiment), but frequently lay egg clutches larger than would be suggested by the function for density-dependent proportional survival (i.e. a clutch size of unity in the clutch size experiments) (Fig. 3).

Another possible explanation is that an ample supply of larval food patches is not found in nature (although fruits usually occur at high local densities) and thus we tested behaviour under conditions for which the flies have not been selected. If the reproductive success of flies is mainly determined by their opportunities for producing clutches (i.e. locating patches), then flies should produce the Lack clutch size (Parker and Courtney, 1984; Godfray *et al.*, 1991). As seen in Fig. 5, productivity (i.e. the number of emerging adults per fruit) increases with egg density. The regression equation in Fig. 5, with $b = 1.062 \pm 0.221$ ($P_{(b)} = 0.001$), suggests that *D. subobscura* larvae engage in contest competition (see Jones *et al.*, 1996) and thus maximum productivity is reached asymptotically. The productivity curve found here is extremely flat and, although maximum productivity is reached at 26 eggs per fruit, 82% of the maximum productivity is achieved at 4 eggs per fruit. If we assume the extreme condition that every single patch is found by wasps and take into account the positively density-dependent mortality found in our experiments, the Lack clutch size would be reached at 4.4 eggs per fruit. For more moderate parasitoid pressures, the productivity curve will fall between the two lines given in Fig. 5, suggesting that flies should lay between 4.4 and 26 eggs per fruit to produce Lack clutch sizes.

However, the optimal clutch size will depend on several factors, such as the threat from parasitoids, the prevalence of competitors in the habitat and the degree of egg or time

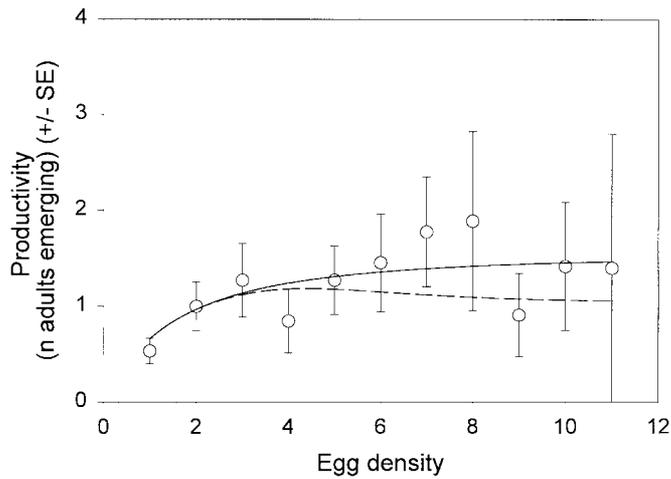


Fig. 5. The density-dependent productivity of *Drosophila subobscura* on *Sorbus aucuparia* fruits. A regression of the type $y = x(1 + (ax)^b)^{-1}$ (see Fig. 3) gives $y = x(1 + (0.529x)^{1.062})^{-1}$, $r^2 = 0.422$, $P = 0.0304$, $n = 11$ (we omitted egg densities where we obtained less than five replicates, as such data points occurred at high egg densities and, because of their larger variation in productivity compared with the survival value in Fig. 3, might inappropriately affect the results of our regression) and is depicted by the solid line. The dashed line results from the incorporation of our results for the density-dependent mortality induced by parasitoids from Fig. 4 into the regression, and the assumption that each fruit is found and searched by a parasitoid.

limitation of flies. Although it is a co-dominant species, *Asobara tabida* is not the only parasitoid species attacking *D. subobscura*, and the density dependence of parasitism might be different in other larval parasitoids. Nevertheless, larval facilitation in reaching spatial refuges was not seen in our experiments (larvae frequently came back to the fly's oviposition site where they could be attacked by wasps) and thus we do not expect to find negatively density-dependent parasitism in other larval parasitoids of *D. subobscura*.

Do potential competitors provide a functional explanation for the aggregation of eggs in *Drosophila*? As Suzuki and Iwasa (1980) have shown, anticipation of competition should reduce the clutch size that a female lays. Consequently, the potential prevalence of competitors cannot explain the large clutch sizes and high egg densities found in our experiments.

If time limitation is more important than egg limitation, flies should lay more than a single egg per fruit. Lifetime egg counts from individual *D. subobscura* flies show that females can lay an average of approximately 12 eggs per day with a huge variation between days and a maximum of around 50 eggs (M. Rohlf, unpublished). If egg complement varies between days, and if resources vary in their availability (e.g. because they are clustered in space and time), we can expect the egg or time limitation to be variable among individual flies. Given the flat form of the productivity curve, we may expect large effects on clutch size with small changes of egg or time limitation in flies, or, alternatively, only weak selection on clutch size or selection for discrimination against previously egg-infested resources, as is frequently found in insects parasitic on plants (Roitberg and Prokopy, 1987) or other insects (Van Lenteren, 1981). The latter would in particular explain the large variation that we have found in fly behaviour, given that flies cannot measure resource quality precisely. A productivity curve like that here would not severely penalize an overestimation of resource quality (Godfray and Ives, 1988), and large behavioural variation in the population might easily be maintained (see Roitberg, 1990). However, these explanations rest on the assumption that eggs are not limiting. Further investigations are needed to elucidate the amount of egg or time limitation of drosophilids under field conditions.

In conclusion, we hope to stimulate a discussion and investigation into what we consider an under-explored field in ecological research. Whereas the effects of egg aggregations for the co-existence of competing species are fairly well understood (Atkinson and Shorrocks, 1984; Shorrocks, 1991; Sevenster, 1996; but see Green, 1986), no functional explanation has been presented to explain why flies produce such aggregations of offspring. In our experiments on clutch size and egg distribution by *Drosophila subobscura* females across fruits of *Sorbus aucuparia*, we tested two hypotheses that are often proposed to explain aggregations. Based on the assumption that flies should maximize offspring survival, we can dismiss what are perhaps the two most likely functional explanations for an aggregative egg distribution of *Drosophila* flies.

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