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Are fitness effects of density mediated by body size? Evidence from *Drosophila* field releases

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ABSTRACT

Question: Does a high larval density decrease adult field fitness by reducing body size?
Hypothesis: Larval density influences capture success by changing adult body size.
Organism: *Drosophila melanogaster* reared under different larval density conditions.
Field site: Eucalyptus woodland lacking soft fruit on which *Drosophila* breed.
Methods: Flies from different density conditions were released at a central point, and then captured on banana bait in a series of experiments. Wing size was measured as a surrogate of body size.
Conclusion: Capture success varied with density, but was not positively related to size in females. Females tended to be caught sooner than males and large females were caught sooner than small females when conditions were cool. Larval density influenced adult field fitness but not necessarily by affecting size.

Keywords: body size, capture success, density, *Drosophila*, field fitness, resource location.

INTRODUCTION

It is well known that in insects larval crowding reduces the body size of adults. This change in adult size is thought to reflect larval competition; in fact, a reduction in the size of a strain/species when cultured together with another strain/species is commonly taken as evidence for intraspecific/interspecific competition (Denno *et al.*, 2000; Lane and Mills, 2003; Sato *et al.*, 2004; Wagner, 2005). A reduction in adult size is often equated with a decrease in adult fitness; there is particularly good evidence in insects for a direct and positive association between size and reproductive fitness when measured as female fecundity (Honek, 2003), although this is not always the case, as demonstrated for male territorial defence (Zamudio *et al.*, 1995).

However, a few studies, mainly with *Drosophila*, have suggested that larval rearing density can influence adult fitness in ways other than via adult size. High larval rearing density decreases adult mass in three species of *Drosophila*, but rearing density only affects

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longevity in one of these species (Baldal *et al.*, 2005), suggesting that longevity effects of density are partly independent of size. In *D. melanogaster*, adult longevity, heat stress resistance, and Hsp70 production tend to be relatively higher when larvae are reared at an intermediate versus low density (Sorensen and Loeschcke, 2001). Selection experiments involving larvae held at different densities for a number of generations indicate changes in feeding behaviour unrelated to adult size (Santos *et al.*, 1997). Waste products like urea and ammonia increase in larval *Drosophila* food as larval density increases, and these products could interfere with metabolic functions in adults independent of any effects on size (Borash and Ho, 2001).

One way of examining the impact of size and larval density on adult fitness of small insects like *Drosophila* is to consider the ability of adults to locate resources. This approach has been used to investigate the association between body size and field performance in parasitoids (Bennett and Hoffmann, 1998; West *et al.*, 1996). Although we are unaware of relevant *Drosophila* studies, field resource location has previously been used in *Drosophila* to examine movement rates (Coyne *et al.*, 1987; Markow and Castrezana, 2000) and the effects of physiological condition and genetic variation on resource response (Hoffmann *et al.*, 1984; Turelli and Hoffmann, 1988).

We assessed the effect of larval rearing density and associated effects on adult size on field resource location in *Drosophila melanogaster*. The following issues were addressed. First, does rearing density influence resource location when flies are released in an area with artificially provided food? We undertook five releases in eucalypt woodland at different temperatures and examined the ability of flies to reach banana bait after release. Second, are effects of rearing density on food location related to effects of body size as measured by wing size? Samples of flies from the density treatments obtained before release were measured for wing size, and treatment effects on capture success were correlated with treatment effects on size. Third, is there an association between capture success and size independent of the density treatments? For three of the five releases we measured wing size of captured flies as well as pre-release samples. These flies were used to assess whether size influenced capture success, and whether size changed with the time of capture.

METHODS

Stocks and culture

We used an isofemale line that was inbred for three generations of sib mating ($F = 0.5$). This reduced the amount of genetic variation among experimental flies, making it potentially easier to detect the effects of environmental factors including density. The line was initiated from an inseminated female collected at Innisfail, Queensland, Australia. To generate flies reared at different larval densities, we used bottles with 50 ml of an agar–sucrose–dead yeast (1.6% w/v, 5% w/v and 6% w/v respectively) laboratory medium containing propionic acid and methyl-*p*-hydroxybenzoate as preservatives. Different numbers of parents (7, 24, or 100 pairs of males and females) were left in the bottles for 24 h at 25°C to generate a range of larval densities reflecting the number of eggs laid. This approach was followed instead of directly counting out larvae onto medium because of the large number of flies required for the field releases. These treatments produced 100–200 offspring (7 pairs), 250–500 offspring (24 pairs), and 1200–1500 offspring (100 pairs) per bottle. Flies from the three density treatments were collected from bottles over the same 1–2 day period. Emerging flies of each density treatment were mixed across bottles (flies were pooled across 12, 7, and 3 bottles for the 24-, 7-, and 100-pair group respectively). Flies were then separated into groups of 100

without anaesthesia (but cooled on ice to slow movement and make counting easier) and held in vials with laboratory medium at 20°C under light until the day of release. Flies were 2–4 days post eclosion (release 1–4) or 5–6 days post eclosion (release 5) at the time they were released in the field.

Releases

The releases were undertaken in an isolated woodland at Kinglake, 50 km north-east of Melbourne. The release site was located more than 5 km from the nearest houses and farms. The woodland consisted of messmate *Eucalyptus* canopy with a shrubby understorey consisting mainly of common heath, pomaderris, and clematis. There were no soft fruit suitable for *Drosophila melanogaster* development in the woodland. Although a few endemic *Drosophila* can be collected from woodland near Melbourne (Parsons, 1982), *D. melanogaster* does not breed in this habitat and the only *D. melanogaster* collected there are likely to be transient migrants.

Flies were tested for their ability to locate fruit in buckets in five separate releases (Table 1) during spring/early summer (November to December) and autumn (April). At the day of release, buckets with freshly cut bananas were placed on a circle with a radius of 32 m, and buckets spaced 10 m apart around the perimeter. The release took place at a central point. The distance between the release point and capture points is similar to the outer capture points in previous designs where baits were placed in a grid and some traps were located closer to the release point (Hoffmann and Turelli, 1985). Buckets were left 2–3 days, except that they were placed on their sides when it rained to avoid flooding of the banana bait.

Flies were released in the afternoon depending on weather conditions (12.00–15.00 h) and their capture started 1 h after release and continued at 1-h intervals. Flies were also collected on the subsequent two days (designated days 1 and 2, as opposed to the day of release which was designated day 0). The overall aim was to obtain three capture samples, which included the first flies arriving at the baits, flies with an intermediate arrival time, and flies that arrived late. However, often only a few flies were captured two days after release. Released flies were marked with Radiant micronized fluorescent dust. To distinguish flies from the density treatments, different colours (blue, pink, yellow, and orange) were used. Each batch of 100 flies was marked with 0.05–1.0 μg of dust in an empty vial by shaking the vial. At the release site, flies were transferred to vials with dust immediately before release. Colours were randomly assigned to the different density classes and changed between releases, and the positions of vials with marked flies were randomized before release. Marking is likely to have only minor effects on fly movement (Turelli *et al.*, 1986).

Table 1. Temperature data and capture periods for the five releases

Release	Average temperature (°C)	Temperature range (°C)	Release to last capture period
1	not known	8.5–20.2	12.00 h on day 0 until 18.00 h on day 2
2	13	9.6–21.7	14.00 h on day 0 until 19.00 h on day 2
3	18.9	10.7–32.3	15.00 h on day 0 until 11.00 h on day 2
4	18.6	6.2–30.1	15.00 h on day 0 until 10.00 h on day 2
5	18.5	11.3–36.6	14.00 h on day 0 until 18.00 h on day 1

Temperature data at the release site were collected with a datalogger (Tinytalk II) and a thermometer recording maximum and minimum temperature. Unfortunately, the datalogger did not function properly during the first release, so only maximum and minimum data are available. Temperatures were low in the first two releases (Table 1) but still exceeded values above the flight threshold of 15°C (Lehmann, 1999) for several hours. In the other releases, temperatures reached 30°C for short periods, particularly in the last release. Fly numbers captured in the traps tended to be higher when temperatures were relatively lower (see below).

Size measurements

Wing size was used as a surrogate for body size, and measured as the centroid size of the right wing according to Hallas *et al.* (2002). After flies were killed with ether, their wings were removed with a fine forceps and placed on a microscope slide between double-sided sticky tape and cover glass. Wing images were captured under a compound microscope with a camera (PixeLink, Vitana Corp.). We placed landmarks on the digital images using tpsDig version 1.2 software developed by F. James Rohlf. Five external landmarks were placed on the ends of longitudinal veins 2, 3, 4, and 5 and on a junction of the auxiliary vein with longitudinal vein 1. Four internal landmarks were placed on both ends of the anterior and posterior cross vein respectively. The centroid size of each wing (the square root of the sum of the squared inter-landmark distances) was computed from all recorded landmarks.

Wings were measured for all flies collected from releases 2, 3, and 4. In addition, we scored size on pre-release samples from all releases. These samples were collected randomly just before release. We scored the wing size of 30–55 males and a similar number of females in each pre-release sample.

Statistical analyses

Statistical analyses were carried out in SPSS for Windows Version 13. All size data were initially checked and confirmed to be normally distributed with Kolmogorov-Smirnov tests.

We assessed effects of the three density treatments on size using a two-way analysis of variance, with sex and density treatment as fixed factors. Because of sex × density interactions in three of the releases (see below), we also tested the effects of density on size of the sexes separately. *Post-hoc* tests (Tukey b) were carried out to determine which treatments differed significantly.

Capture data were initially treated as categorical and analysed separately for each release. To examine the impact of density on capture success, flies of each sex were separated into two groups (captured and not captured). To estimate the number of males and females in the sample that was not captured, we assumed a 1 : 1 sex ratio (1000 flies of each sex per treatment released). This assumption was experimentally validated by finding no deviations from 1 : 1 in the pre-release sample (data not presented). We then used log linear models to assess the interaction between density treatment, capture success, and sex. This analysis assumes that each fly represents an independent data point for a particular density treatment within a release. Log linear models were selected based on both backward elimination and forward selection. Where there were interactions between capture and density treatment, we undertook contingency analysis for each sex to investigate further the interaction between these factors. To assess whether density and sex influenced the number of flies

captured on the different days, a second set of log linear analyses was performed, considering only data from the capture samples. These log linear models considered interactions between density treatments, day of capture, and sex in each release, with the assumption that captured flies from different treatments and captured on different days represented independent data points.

Two additional analyses were undertaken to test for consistent patterns across releases. In the first analysis, non-parametric Friedman tests were used to compare density treatments across releases. In one test, we assessed whether the proportion of flies captured within a release was consistently different between the density treatments. In the other test, we assessed whether the proportion of flies captured on the first day flies were collected (day 0 or day 1) differed between density treatments. Male and female data were analysed separately.

In the second analysis, we considered whether differences in wing size between the density treatments could be correlated with differences in capture success. To test this, we considered the 24-pair treatment as a standard. We then subtracted the 7-pair values (for mean wing size or capture success) from the 24-pair values and plotted the body size difference against the difference in capture success for all five releases (with each release providing a data point). If size influenced treatment differences in capture success, we expected a positive association between these variables. A similar comparison was carried out between the 24-pair and 100-pair treatments. Spearman rank correlation coefficients were computed to test the significance of this association across releases. We also tested for consistent differences between the 24-pair/100-pair and 24-pair/7-pair comparisons across releases for size and capture success, using Wilcoxon paired-sample tests.

Analyses of variance were carried out to test if size differed between capture and pre-release samples, and whether differences depended on the density treatments (capture $\times$ density interactions). Analyses of variance were performed separately for the two sexes and for the three releases in which data on wing size of captured individuals were collected. We undertook separate analyses of variance on the captured samples to determine whether there was a difference in wing size across the days of capture, and if any differences depended on density.

RESULTS

Size of pre-release samples

The 100-pair treatment produced the smallest flies, with the exception of males in release 2 (Fig. 1). The effect of density was significant by analysis of variance in four of the five releases, regardless of whether the sexes were analysed together or separately (Table 2). A sex $\times$ density interaction was also evident in three of the five releases. For females, the 24-pair treatment produced larger flies than the 100-pair treatment in four releases, but not in release 3. Females from the 7-pair treatment were significantly larger than those from the 100-pair treatment in two releases, while in the other three releases there was no difference between these treatments. Males showed a different pattern to females in several releases, accounting for the significant interaction terms in the analyses of variance. The 7-pair treatment produced the largest males except in release 3. Males from the 7-pair treatment were significantly larger than those from the 100-pair treatment in four releases, and significantly larger than those from the 24-pair treatment in two releases (Fig. 1). Overall, the

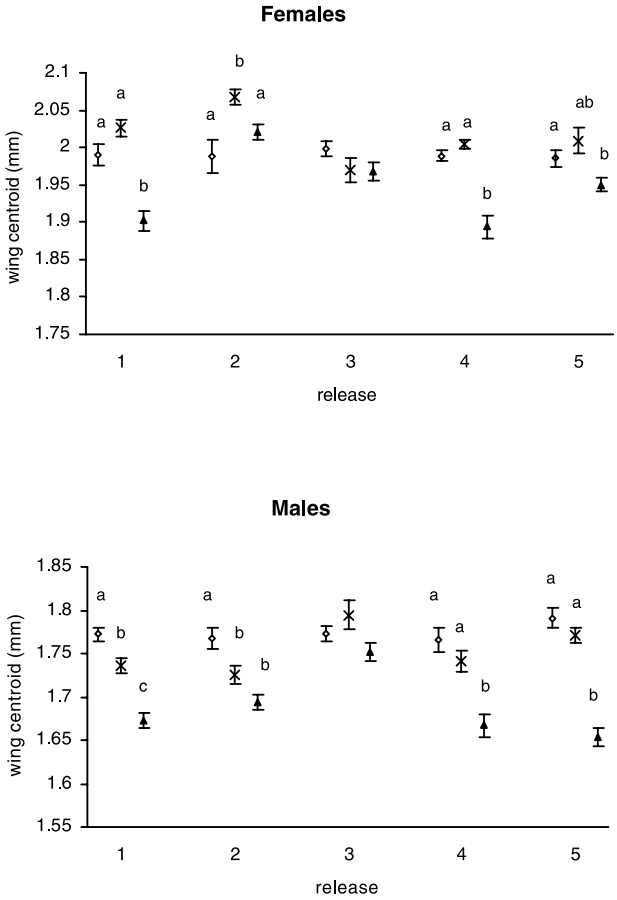

Fig. 1. Wing size in pre-release samples of flies reared at different densities. ◇, 7-pair treatment; ×, 24-pair treatment; ▲, 100-pair treatment. Letters indicate significant differences by *post-hoc* Tukey b-tests. Error bars are standard errors.

density treatments succeeded in producing flies for releases with different wing size in some releases but not in others.

Effect of density treatment on capture success

More flies were captured in the first two releases carried out under cooler conditions than in the other releases that included warm periods (Fig. 2). Log linear models selected based on backward elimination led to different models for the releases (Table 3). In releases 1 and 5, the capture × sex term was significant. More males than females were captured in release 1, whereas the reverse occurred for release 5 (Fig. 2). In release 2, there was a three-way interaction between sex, density treatment, and capture success. Contingency analyses indicated significant associations between capture and density treatment in females ($G = 15.25$, d.f. = 2, $P < 0.001$) but not in males ($G = 1.35$, d.f. = 2, $P = 0.51$). Females reared under low-density conditions had a relatively higher capture success, with the 24-pair treatment being

Table 2. Results of analyses of variance testing the effects of density treatments and sex on wing centroid size

	Effect	Release 1	Release 2	Release 3	Release 4	Release 5
Both sexes	Sex (d.f. = 1)	4.0283***	3.7787***	2.7574***	1.8625***	3.8825***
	Density (d.f. = 2)	0.2595***	0.0269**	0.0159	0.1124***	0.2237***
	S × D (d.f. = 2)	0.0321**	0.0525***	0.0152	0.0067	0.0553***
	Error (d.f.)	0.0054 (262)	0.0040 (179)	0.0071 (259)	0.0030 (171)	0.0060 (262)
Females	Density (d.f. = 2)	0.1682***	0.0409***	0.0127	0.0620***	0.0436**
	Error (d.f.)	0.0072 (130)	0.0045 (88)	0.0073 (129)	0.0024 (104)	0.0076 (142)
Males	Density (d.f. = 2)	0.1192***	0.0410***	0.0184	0.0513***	0.2104***
	Error (d.f.)	0.0036 (132)	0.0035 (91)	0.0069 (130)	0.0040 (67)	0.0040 (120)

Note: Analyses of variance were computed for both sexes combined and for the two sexes separately.
$^{**}P < 0.01$; $^{***}P < 0.001$.

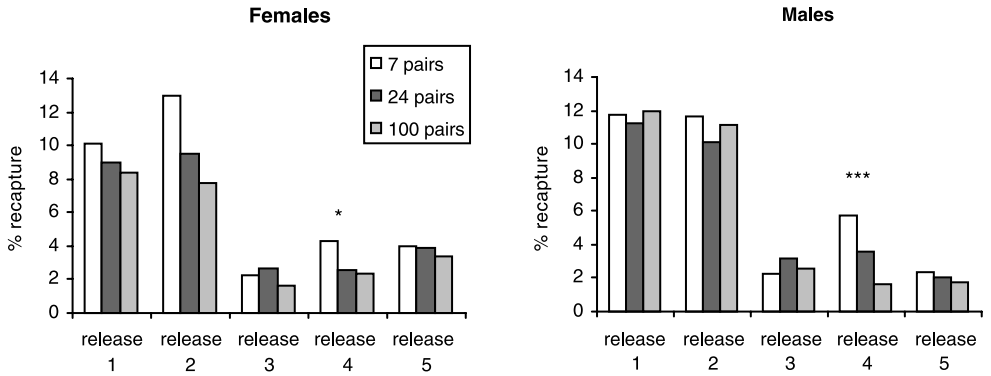

Fig. 2. Percentage of released flies from the different treatments captured in the five releases. Asterisks indicate releases where treatments differ significantly by a contingency test ($^{*}P < 0.05$; $^{***}P < 0.001$).

intermediate (Fig. 2). In release 3, there were no interactions between any of the variables, reflecting the fact that capture success was unrelated to density or sex, although numbers caught in this release were low. For release 4, the capture × sex interaction was included in the log linear model (Table 3). Capture success was relatively higher for flies reared in the low-density treatment (Fig. 2), and this difference was significant in a contingency analysis for both females ($G = 7.49$, d.f. = 2, $P = 0.02$) and males ($G = 25.30$, d.f. = 2, $P < 0.001$).

We assessed the effects of density on capture success across all releases with Friedman rank tests. These indicated a significant overall difference between density treatments for the females ($X^2 = 8.4$, d.f. = 2, $P = 0.015$) but not the males ($X^2 = 1.2$, d.f. = 2, $P = 0.549$).

To examine the association between size differences and capture success of the density classes, we plotted the differences between density treatments (24-pair minus either 7-pair or

Table 3. Interaction terms included in log linear models fitted to capture data from each release

Release	Term	d.f.	ΔG
Pre-release versus capture samples			
1	Capture × sex	1	10.07**
2	Capture × sex × density	2	6.19*
3	–	–	–
4	Capture × density	2	29.25***
5	Capture × sex	1	16.24***
Variability within capture samples			
1	Density × day	2	13.79***
2	Sex × day	1	40.22***
	Density × day	2	17.326***
	Sex × density	2	10.43**
3	Day × sex	2	40.22***
4	Day × sex	2	24.30***
5	Day × sex	1	7.81**
	Day × density	2	31.59***

Note: Two sets of comparisons were undertaken, involving pre-release versus capture samples (testing capture, sex, and density effects) and capture samples (testing density, day, and sex effects). The change in the likelihood ratio (ΔG) and probability when this term was excluded from a saturated model (pre-release/capture comparison, release 2) or a model with all two-way interactions (all other comparisons) is given.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

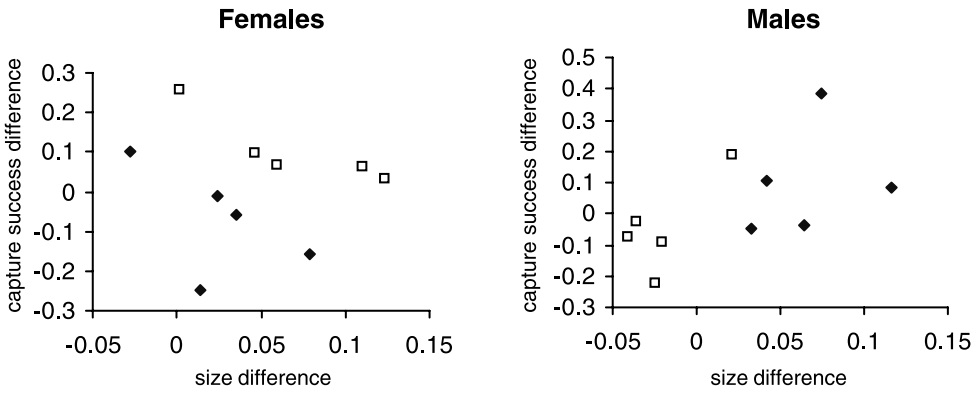

Fig. 3. Difference in size between two density treatments (◆, 24-pair minus 7-pair; □, 24-pair minus 100-pair) in a release plotted against the difference in the proportion of flies captured.

100-pair flies for both size and capture success). In females, these plots provided evidence for a negative rather than the anticipated positive association between the size difference and capture probability of the treatments (Fig. 3). The negative association was significant in the 24-pair and 100-pair comparison (Spearman rank correlation, $P < 0.001$) but not for the 24-pair and 7-pair comparison ($P = 0.30$). The lack of a positive association between

size and capture success is also evident from Figs. 1 and 2. For instance, in release 2 females from the low-density treatment had a relatively higher capture success, but females from this density treatment were not larger than those from the 100-pair treatment. The comparison plotted in Fig. 3 also indicates that the difference in capture success between the 24- and 7-pair treatments was smaller than between the 24- and 100-pair treatments. This was significant by a Wilcoxon paired-sample test ($z = 2.023$, $P = 0.043$).

For the males, size differences generated by the density treatments were positively associated with capture success (Fig. 3), but associations were not significant by Spearman rank correlation tests for either the 24-pair/7-pair or the 24-pair/100-pair comparisons. Figure 3 also indicates that the size difference between the 24- and 7-pair treatments was larger than between the 24- and 100-pair treatments, which was significant by a Wilcoxon paired-sample test ($z = 2.023$, $P = 0.043$). The difference in capture success between these comparisons did not differ significantly ($z = 0.944$, $P = 0.345$).

Distribution of flies within capture samples

We tested whether density treatment influenced the pattern of capture across days. For release 1, flies from the 7-pair treatment were relatively more common on the first day but were under-represented on the second day (Fig. 4). Log linear models indicated a density × day interaction for this release (Table 3). In release 2, the density × day interaction was again significant and reflected a similar capture pattern for the 7-pair treatment. In this release, more males than females were caught on the first day, a pattern that was reversed on the second day, resulting in a significant day × sex interaction. In release 3, there were no density effects, but more females than males were captured early in the release and this pattern was sharply reversed later (Fig. 4), resulting in a day × sex interaction. In release 4, relatively more males than females were caught early in the release, resulting in a day × sex interaction. In the final release, this pattern was repeated. In addition, a relatively high number of flies captured on day 1 were from the 24-pair treatment, whereas those caught on the day of the release (day 0) were more likely to be from the 7-pair treatment, resulting in a day × density interaction (Table 3).

These results suggest density and sex effects on patterns of capture over time, particularly for females and flies from the 7-pair treatment. To test for overall treatment differences over the five releases, we used Friedman rank tests. These indicated that the proportion of flies caught in the first collection differed significantly between the density treatments for females ($X^2 = 7.6$, d.f. = 2, $P = 0.022$) but not for males ($X^2 = 1.6$, d.f. = 2, $P = 0.449$).

Size in capture samples

Mean size of the females is plotted in Fig. 5 separately for the different days of capture because capture day influenced size (see below). Females in the capture samples were on average smaller than in the pre-release samples except in release 3 (Fig. 5). In the analyses of variance, size differed significantly between the pre-release and capture samples for two of the three releases, and there was no interaction between density treatments and capture (Table 4). Density treatments had a significant effect on size of females in two releases, consistent with patterns for the pre-release samples (cf. Table 2).

Males from the pre-release sample were relatively larger than those from the capture sample in release 4 (Fig. 5) but the samples did not differ significantly in the other releases

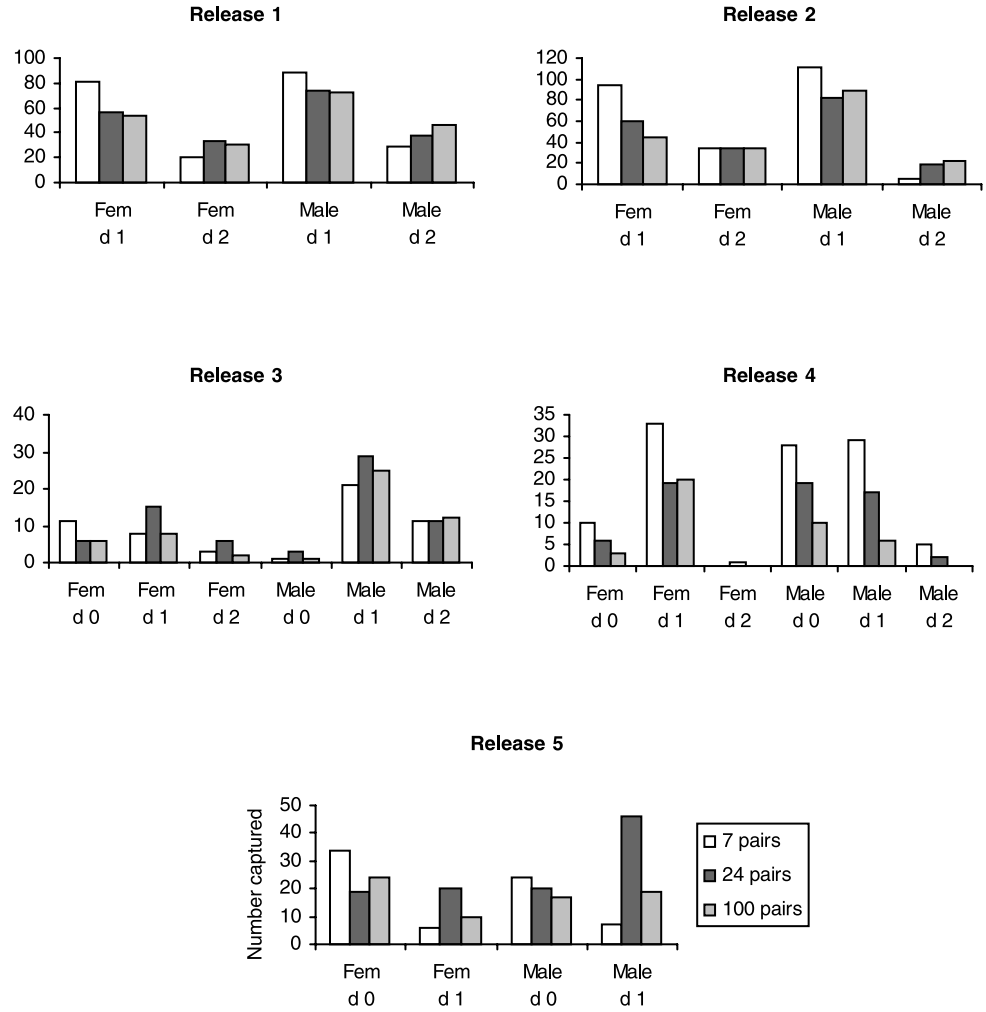

Fig. 4. Number of flies from the three density treatments caught on different days in the five releases. Day 0 (d0) represents captures on the day of release, day 1 (d1) and day 2 (d2) represent captures on ensuing days.

(Table 4). There was a significant interaction between density and capture in release 4, because captured males from only two of the three density treatments were smaller than those from the pre-release sample (Fig. 5). Density influenced male size in all three releases, including release 3 where a density effect had not been previously detected when based only on the pre-release sample (cf. Table 2). The 100-pair treatment tended to produce consistently smaller flies (Fig. 5).

As well as investigating differences between pre-release and capture samples, we also considered size variation among samples captured on different days. Females from the first day of capture were relatively larger in two of the releases, but the opposite trend was observed in release 4, which took place in warmer conditions (Fig. 5). In the analyses of variance, capture day had a significant effect on size in two releases, and was marginally

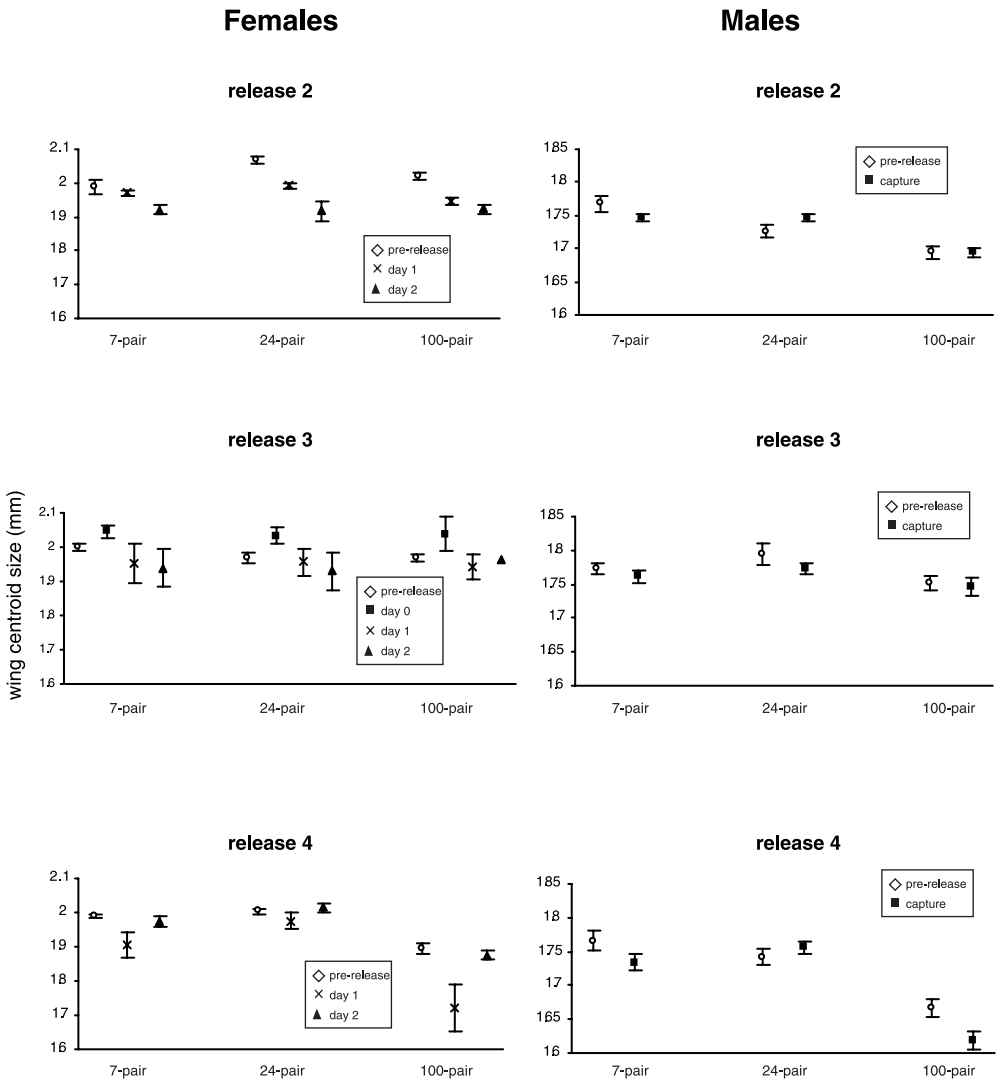

Fig. 5. Wing size of flies caught on different days in the three releases for which capture samples were measured. For females, capture days are presented separately because the size of females captured on different days varied. For males, data were pooled across days because male size did not differ significantly between days. Pre-release sample means are also included for comparison. Error bars are standard errors.

non-significant in release 3 (Table 4). Male size did not vary between the capture days as reflected by the lack of significant effects of day in the analyses of variance (Table 4).

DISCUSSION

To what extent have our questions been answered? The first question was concerned with whether rearing density can influence the ability of flies to locate food resources, and the

Table 4. Results of analyses of variance testing the impact of capture, capture day, and culture density on wing centroid size of females and males

			Females			Males		
	Effect	d.f.[a]	MS	*F*	*P*	MS	*F*	*P*
Pre-release versus captured samples								
Release 2	Capture	1	0.2534	51.59	<0.001	0.0000	0.00	0.957
	Density	2	0.0689	14.02	<0.001	0.0950	29.26	<0.001
	Interaction	2	0.0143	2.92	0.056	0.0089	2.73	0.067
	Error	319/345	0.0049			0.0032		
Release 3	Capture	1	0.0009	0.09	0.766	0.0127	2.07	0.151
	Density	2	0.0118	1.11	0.331	0.0274	4.47	0.012
	Interaction	2	0.0005	0.05	0.954	0.0015	0.24	0.788
	Error	193/284	0.0106			0.0061		
Release 4	Capture	1	0.0182	4.81	0.030	0.0146	4.00	0.047
	Density	2	0.1721	45.36	<0.001	0.1372	37.66	<0.001
	Interaction	2	0.0060	1.58	0.209	0.0122	3.36	0.038
	Error	164/142	0.0038			0.0036		
Captured samples only								
Release 2	Day	1	0.0636	13.33	<0.001	0.0015	0.46	0.498
	Density	2	0.0033	0.68	0.506	0.0319	10.04	<0.001
	Interaction	2	0.0055	1.16	0.315	0.0022	0.68	0.505
	Error	228/251	0.0048			0.0032		
Release 3	Day	2	0.0491	2.90	0.063	0.0041	0.73	0.482
	Density	2	0.0003	0.02	0.982	0.0043	0.77	0.464
	Interaction	4	0.0006	0.04	0.997	0.0012	0.22	0.926
	Error	58/148	0.0169			0.0056		
Release 4	Day	1	0.0676	12.96	<0.001	0.0044	1.31	0.277
	Density	2	0.1078	20.67	<0.001	0.0769	22.91	<0.001
	Interaction	2	0.0095	1.83	0.170	0.0014	0.43	0.732
	Error	57/70	0.0052			0.0034		

Note: Analyses of variance were undertaken to compare the pre-release samples and the entire capture samples, or to compare the samples captured on different days.

[a] For error term, first d.f. is for females, second d.f. is for males.

results indicate that they clearly can. Females from the low-density treatment had a relatively higher capture success than those from the other two treatments, while those from the highest density treatment had the lowest success. These differences were consistent across the releases and independently significant in two of the releases. For males, the picture was less clear-cut. In one release males reared at a low density were more likely to be captured, but in the other releases there was no consistent pattern. Thus overall density effects were more apparent and more consistent in females than in males. These findings indicate that density influences the field behaviour of insects and provide further support for density

effects on a range of laboratory-defined traits, including reproductive output (Honek, 1993), stress resistance (Bubli *et al.*, 1998; Sorensen and Loeschcke, 2001), and longevity (Baldal *et al.*, 2005).

The second question asked whether rearing density effects are mediated through size, and here the results did not match predictions. We generated flies that differed in size in some releases but not in others, likely to reflect the fact that we did not precisely control for larval density, although food quality variation might also have played a role. This variability was used to test if those releases where density conditions influenced capture success were the same as those where density conditions influenced size. For females, we observed differences in capture success in releases where there were no differences in wing size between treatments and vice versa. Moreover, there was a negative relationship between the differences in capture success and in size between density treatments, rather than the predicted positive association. Only in males was there any suggestion of a positive association between treatment differences in size and capture success. Thus, as in the case of longevity effects investigated in *Drosophila* (Baldal *et al.*, 2005), there is not necessarily an association between density effects and size effects. In our case, this applies to size as measured by the wing size of flies: it is possible that density effects could be mediated by other size-related effects, such as wing shape or the ratio of wing to thorax length, which is thought to influence flight ability in *Drosophila* (Petavy *et al.*, 1997). Density effects might also be mediated by metabolic effects, perhaps as a consequence of the high level of waste products present in medium with a high larval density (Borash and Ho, 2001), or through effects on body mass, which can be influenced independently of size in insects (Strobbe and Stoks, 2004).

The third question relates to size effects independent of density, and we did find effects of wing size on capture success within the density treatments in two (females) or one (males) of the three releases. The reason why captured flies were smaller than released flies is not clear. Within releases there was a tendency for larger flies to be captured early when conditions were cool and later when conditions were warm. Perhaps size effects on capture success depend on the thermal environment, although additional releases would be required to test this conjecture. It is also possible that large flies rapidly moved away from release sites when conditions were cooler, even though there were no resources away from the experimental site. These issues will only be resolved with trap lines placed at different distances from a release point. Release experiments on parasitoids have indicated that large size increases the ability of females to reach oviposition sites (West *et al.*, 1996; Bennett and Hoffmann, 1998). A complication is that size differences between pre-release samples and capture samples can depend on when parasitoids are captured (Kolliker-Ott *et al.*, 2004) and we also observed a shift in size over time in the capture sample.

In the experimental design we employed in these releases, there is an underlying assumption that the flies captured at buckets have a relatively higher fitness than those that are not retrieved. Failure of flies to be captured at bait may indicate that flies did not survive or moved away from the experimental site. In release–capture experiments where a rectangular grid is used to capture flies, relatively higher numbers of flies are always retrieved at bait stations near the release site (Hoffmann and Turelli, 1985). The distance that we used in the experiments ensured that flies had to move more than 30 m to the capture station, which is further than the distance typically used in release–capture studies with *Drosophila*. This may explain why the proportion of flies retrieved at the baits was low compared with other studies (Hoffmann and Turelli, 1985), representing less than 12% of the flies released, particularly in the warm temperature releases.

The field design provides one estimate of the field fitness of *Drosophila*. The design only tests the ability of flies to locate feeding and breeding resources in an environment where these are unlikely to exist. In this sense, we are measuring one component of fitness in a species that breeds in ephemeral resources. Some aspects of adult field fitness are not considered, such as mating success and reproduction once flies reach breeding resources. Nevertheless, we feel that this design represents a valuable addition to the laboratory assays that are normally used by *Drosophila* researchers to evaluate fitness.

Larval crowding represents only one way that size variation can be generated in *Drosophila* and other insects. Other ways of generating flies with different sizes include developmental temperature (Karan *et al.*, 1998), altered nutrition (Thomas, 1993), and developmental manipulation (Leevers and McNeill, 2005). While these different methods all influence size, their effects on fitness are likely to vary. For instance, Zamudio *et al.* (1995) reared flies under cool (18°C) and warm (25°C) conditions and then tested the territorial success of males at different temperatures. Those reared at 25°C were smaller but were more successful in territorial encounters. Yet when flies are reared at the same temperature, larger males tend to win territorial encounters (Hoffmann, 1987), suggesting that effects of developmental temperature on size are different to factors that generate phenotypic variation in size.

In conclusion, release–capture experiments showed that larval rearing density influenced the ability of flies to locate food resources in the field. While density effects varied between releases, there was a tendency for females from low-density treatments to be more successful in locating resources, particularly early in a release. For females, density effects on resource location were not mediated via body size, although smaller females tended to be caught more frequently, particularly late in releases.

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