

# Breeding structure of *Drosophila buzzatii* in relation to competition in prickly pears (*Opuntia ficus-indica*)

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**Summary** – Rotting *Opuntia ficus-indica* fruits (prickly pears) are used as breeding sites for up to four *Drosophila* species (*D melanogaster*, *D simulans*, *D buzzatii* and *D hydei*) in southern Spain. A field experiment showed that the larvae of *D buzzatii* are resource limited in *Opuntia* fruits available for oviposition for 108 h. Experimental fruits infested with *D* larvae were divided into two halves; the larvae in one half were allowed to develop normally, while those in the other half were provided with extra food. Approximately five times as many *D buzzatii* emerged from the supplemented as from the control halves, and the flies emerging from the supplemented halves were, on average, larger than those emerging from the control halves. *F*-statistics were estimated from allozyme data for the *D buzzatii* flies. The values obtained from the supplemented halves, coupled with computer simulations to compare these estimates with the expected values generated by a limited number of mating pairs contributing progeny to a fruit, suggest an effective size of about 30 individuals. Even though 95% bootstrap confidence intervals for  $F_{IS}$  estimates comparing the supplemented and control halves do not overlap, computer simulations suggest that we cannot support the hypothesis that selection is acting on allozyme variation.

**body size / cactophilic *Drosophila* / competition / density-dependent mortality / population structure**

**Résumé** – Structure génétique des populations de *Drosophila buzzatii* en situation de compétition dans les figues de barbarie (*Opuntia ficus-indica*). Les fruits pourris d'*Opuntia ficus-indica* (figues de Barbarie) sont utilisés comme sites de reproduction par quatre espèces de drosophiles (*D melanogaster*, *D simulans*, *D buzzatii* et *D hydei*) dans le sud de l'Espagne. Une expérimentation sur le terrain a montré que les larves de *D buzzatii* ont des ressources limitées dans les fruits d'*Opuntia* disponibles pour la ponte pendant 108 h. Des fruits expérimentaux infestés de larves de drosophiles ont été divisés en deux moitiés : dans la première, les larves ont pu se développer normalement et, dans la seconde,

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on a ajouté de la nourriture. À peu près cinq fois plus de *D buzzatii* sont sorties des moitiés complémentées en comparaison aux moitiés de référence, et les mouches sortant des moitiés complémentées ont été en moyenne plus grandes que celles sortant des moitiés de référence. Des statistiques  $F$  ont été estimées à partir de données sur allozymes pour les mouches *D buzzatii*. Les valeurs obtenues à partir des moitiés supplémentées, couplées avec des simulations sur ordinateur pour comparer ces estimées avec les valeurs espérées générées par un nombre limité d'accouplements contribuant au peuplement d'un fruit suggèrent un effectif efficace d'environ 30 individus. Même si les intervalles de confiance de  $F_{IS}$  donnés par la méthode de bootstrap pour les moitiés supplémentées et de référence ne se recouvrent pas, les simulations ne permettent pas d'appuyer l'hypothèse selon laquelle la sélection s'exerce sur la variation allozymique.

taille / drosophile cactophile / compétition / mortalité / structure de population

## INTRODUCTION

Populations of many organisms, particularly insects, are subdivided in the sense that females lay eggs in discrete and ephemeral resources, each used as a breeding site by a small number of individuals (Heed, 1968; Jaenike and Selander, 1979; Shorrocks, 1982; Brncic, 1983; Lacy, 1983; Hoffmann et al, 1984; Santos et al, 1989; Thomas and Barker, 1990; Santos, 1997). A strong motivation to study the effects of such a population structure relates to the pervasive idea that environmental heterogeneity – arising because selection proceeds in different directions in different places, because there are complementary interactions among genotypes, or because there is an aggregated distribution of eggs over patches – can maintain genetic heterogeneity (Levene, 1953; Hoffmann and Nielsen, 1985; Hedrick, 1986; Gillespie and Turelli, 1989; Gillespie, 1991; Dytham and Shorrocks, 1992, 1995). A basic ingredient in most genetic models is the existence of crowded conditions within patches (ie, selection is 'soft', meaning that density regulation occurs within each patch separately). If competition is absent, environmental heterogeneity might be irrelevant to explain genetic variation.

The presence of competition in natural populations of *Drosophila* has been inferred in several cases (eg, Fellows and Heed, 1972; Atkinson, 1979; Prout and Barker, 1989), but a clear experimental demonstration was first provided by Grimaldi and Jaenike (1984). These authors collected mushrooms infested with larvae and divided each mushroom into two; the larvae in one half were allowed to develop normally, while those in the other half were provided with extra food (see also Jaenike and James, 1991). They showed that there is density-dependent mortality in natural populations, and that flies emerging from halves of supplemented mushrooms are larger than flies emerging from control halves. An important conclusion to be obtained from this experiment is that in natural populations of *Drosophila* there is the opportunity for selection (Crow, 1958; Arnold and Wade, 1984). From an evolutionary perspective, however, the important point is not to show that there is opportunity for selection, but that selection does indeed differentially affect the various genotypes.

We report here an experiment designed to investigate the incidence of competition in *Opuntia ficus-indica* fruits (prickly pears) in the field, together with an analysis of genetic diversity for the *Drosophila buzzatii* (Patterson and Stone) flies that emerged from natural substrates. Adults of this species have been reared from

rotting *Opuntia* cladodes, which are not significantly utilized as breeding sites by other drosophilids in the Old World (at least during the summer months, Santos et al, 1988, 1992). In contrast to this, the *Opuntia* fruits can be exploited by other *Drosophila* species in southern Spain. These fruits are sweet, fleshy, frequently visited by *Drosophila* adults after falling from the plant, and very easy to manipulate experimentally. Although they are individually small (from approximately 30 to 90 g of wet weight), 'en masse' they can form habitats of considerable size. We do not have an estimate of the relative contributions of *Opuntia* fruits and cladodes to the total population density of *D buzzatii*, but during the fruit season (from August to November in southern Spain) it is quite likely that a significant proportion of *D buzzatii* flies come from *Opuntia* fruits. We investigated the allozyme genotypes of *D buzzatii* emerging from the fruits, and obtained estimates of *F*-statistics. Because under field conditions we are never sure of what fraction of the genetic differentiation is attributable to drift (founder events of individual patches) or to selection, the flies raised from halves of supplemented fruits (= 'non-limited resource', see below) were used to obtain empirical distributions of *F*-statistics likely to be due to drift. Measures of inbreeding were then used to estimate the effective number of parents contributing gametes to each fruit, and to see whether or not there is genetic differentiation between breeding sites as a result of selection.

## Materials and methods

### *Description of collections*

Samples were collected in September 1993 from a disused *Opuntia ficus-indica* plantation (Carboneras, SE Spain), described in detail elsewhere (Ruiz et al, 1986). At that time of the year there are abundant *Opuntia* fruits which are exploited by *D melanogaster*, *D simulans*, *D buzzatii*, and *D hydei*.

On 3 September, 300 undamaged mature fruits were harvested from the *Opuntia* stems, labelled with coloured bands, and placed at random in the experimental area on 4 September after cutting a small slice at the top to allow for oviposition by *Drosophila* females. After various periods of time in the field these fruits were recollected and placed separately in jars on a bed of sand, covered with gauze. After 24 h, 30 labelled fruits were collected. After 48 h, another 30 fruits were collected and divided in half longitudinally. One half of each fruit ('control' half) was left untreated while half of a fresh, uncolonized fruit, was added to the other 'supplemented' ('= non-limited resource') half. This approximately doubled the wet mass of food available to larvae. The same procedure was followed for an additional sample of 30 fruits collected after 72 h. Finally, on 9 September we randomly collected 124 fruits out of the remaining 210 fruits that had been left in the field for 108 h. Control and supplemented halves were produced for 63 of those fruits, whereas the remaining 61 were placed into separate jars without cutting. These fruits served as the control for the cutting treatment.

The experimental fruits were kept at room temperature (22–27 °C) in the makeshift laboratory near the field site and checked regularly for emergent adults. From the time of first adult emergence (13 September), all jars were examined daily and emerged adults were fixed in a 3:1 mixture of alcohol and glycerol except

*D buzzatii* flies, which were kept alive in vials containing 5 mL of standard cornmeal-agar-yeast food.

*D melanogaster* and *D simulans* males were distinguished by the differences in their external genitalia (Sturtevant, 1919). No attempt was made to distinguish between females of these two species and their numbers were grouped together into a single class. For each species that emerged from the two halves of the 63 fruits that remained in the field for 108 h, the wing length of up to five males per collection was measured (see Leibowitz et al, 1995). Wing length was used as an index of adult body size because it is a more convenient measure when flies can be killed. The average wing length from each control and supplemented half fruit was calculated by weighting the mean of each collection by the number of males of each species in that collection.

### ***Allozyme electrophoresis***

From most of the 108 h *Opuntia* fruits that yielded *D buzzatii* flies, a random sample of individuals was assayed for four polymorphic enzyme loci (*Est-2*, *Aldox*, *Pept-2* and *Adh-1*). Details of the electrophoretic techniques, allele nomenclature [standardized following Barker and Mulley (1976), and Barker et al (1986)], chromosome mapping, and gametic associations between these loci and between them and the polymorphic inversions in the population of Carboneras, are given elsewhere (Quezada-Díaz et al, 1992; Quezada-Díaz, 1993; Betrán et al, 1994). Briefly, *Est-2* segregates for five alleles, and the other three loci segregate for two alleles each. *Est-2* and *Aldox* are linked to the inversions on the second chromosome, while *Pept-2* is outside the inverted fragments. There are strong linkage disequilibria (sensu Lewontin and Kojima, 1960) between alleles of *Est-2* and *Aldox* with the second chromosome arrangements. Thus, alleles *Est-2<sup>a</sup>* and *Est-2<sup>b</sup>* are segregating within the gene arrangements *2st* and *2j*, with the former allele at higher frequency in *2st* and the latter in higher frequency in *2j*. Allele *Est-2<sup>c+</sup>* is fixed in the gene arrangement *2jq<sup>7</sup>*, and alleles *Est-2<sup>c</sup>* and *Est-2<sup>d</sup>* are only present in the inversion *2jz<sup>3</sup>*. Allele *Aldox<sup>b</sup>* is associated with *2st*. *Adh-1* is located on the third chromosome which lacks polymorphic inversions (Labrador et al, 1990).

### ***Statistical analyses for the allozyme data***

Analyses of allelic frequencies and the calculation of *F*-statistics using the methods of Weir (1990) were accomplished with the GENEPOP (v. 1.2) population genetics software (Raymond and Rousset, 1995).

Associations among alleles at two loci were measured by the composite digenic disequilibrium coefficient  $\Delta_{AB}$  (Weir and Cockerham, 1989; Weir, 1990). Alleles other than *Est-2<sup>a</sup>* were grouped together into a single class. This coefficient is equal to zero if the allelic state at one locus is not correlated with that at another.

## RESULTS

### *Evidence of competition in natural substrates*

A total of 34 745 individuals of the four *Drosophila* species emerged from the experimental fruits of *Opuntia ficus-indica* (39.6% *D melanogaster*, 54.4% *D simulans*, 4.3% *D buzzatii* and 1.6% *D hydei*). Table I shows their average numbers per fruit, together with Wilcoxon matched-pair signed-rank tests (Siegel and Castellan, 1988) comparing the number of flies in each half. After 108 h in the field, approximately five times as many *D buzzatii* emerged from the supplemented as from the control halves (only 187 *D buzzatii* emerged from the control halves, whereas 845 emerged from the supplemented halves). A potential problem with 'the cutting treatment' might be that, for any reason (eg, control halves dried-out quicker), a lower number of adult flies emerged from the control fruits. A comparison of the average number of males and females (*D melanogaster*/*D simulans* females were pooled) of *Drosophila* species that emerged from the 108 h whole fruits with twice as many emerging from the 108 h control halves suggests that this is probably not the case here (Wilcoxon-Mann-Whitney tests ranged from  $z = 0.03$ ,  $P = 0.972$ ; for *D melanogaster* males, to  $z = 1.18$ ,  $P = 0.236$ ; for *D simulans* males). Except for *D hydei*, the Spearman rank correlations between the emergence numbers were positive and statistically significant for all pairs of species in the 108 h supplemented halves. However, in both the 108 h control halves and 108 h whole fruits the *D buzzatii*-*D melanogaster* and *D buzzatii*-*D simulans* rank correlations were very low and statistically non-significant, probably owing to the increase in mortality suffered by *D buzzatii*.

The only differences in size distributions between flies emerging from the 108 h control and supplemented halves were found for *D buzzatii* (table II). Because up to five males per fruit per collection were measured for each species (see *Material and methods*), in the 108 h control halves we had an index of body size for most of the *D buzzatii* males that emerged. Therefore, we could carry out a multiple regression analysis of the effect of each species' density (estimated as the number of males that emerged in a given fruit) on the individual wing length of each *D buzzatii* male. Forward stepwise regression coefficients were statistically significant for *D buzzatii* ( $\beta_{\text{intra}} = -0.018$ ,  $P = 0.008$ ), *D simulans* ( $\beta_{\text{inter}} = -0.004$ ,  $P < 0.001$ ) and *D melanogaster* ( $\beta_{\text{inter}} = 0.002$ ,  $P = 0.006$ ), the negative regression for *D buzzatii* suggesting that intraspecific competition is occurring within the breeding sites. The negative correlation between the wing length of *D buzzatii* and the number of *D simulans* might also indicate the occurrence of interspecific competition, but some care must be taken with such an interpretation because it is possible that conditions that enhance the numbers of *D simulans* may adversely affect the body size of *D buzzatii*. Conversely, some conditions may enhance the numbers of *D melanogaster* and the body size of *D buzzatii*, which could explain the highly significant positive correlation found between both variables.

Figure 1 shows the number of male flies that emerged from the 108 h control and supplemented fruits through time. It is obvious that *D melanogaster* and *D simulans* have shorter development times than *D buzzatii* and *D hydei*, which clearly suggests that they would always be at a competitive advantage at high larval densities (see *Discussion*).

**Table I.** Average numbers of adult flies per fruit ( $\pm$  SE) that emerged from *Opuntia ficus-indica* fruits left in the field for different periods of time.

Species <sup>a</sup>	24 h		48 h		72 h		z
	Whole fruit	Control half	Control half	Supplemented half	Control half	Supplemented half	
♂♂ Dm	1.73 $\pm$ 1.25	7.43 $\pm$ 3.53	6.07 $\pm$ 2.46	0.51 <sup>ns</sup>	12.17 $\pm$ 4.48	14.93 $\pm$ 6.33	0.65 <sup>ns</sup>
♂♂ Ds	9.17 $\pm$ 2.46	10.80 $\pm$ 2.60	9.23 $\pm$ 2.79	1.44 <sup>ns</sup>	25.48 $\pm$ 6.98	24.97 $\pm$ 5.27	0.01 <sup>ns</sup>
♂♂ Db	0.37 $\pm$ 0.31	0.30 $\pm$ 0.24	0.17 $\pm$ 0.14	1.07 <sup>ns</sup>	0.69 $\pm$ 0.32	1.17 $\pm$ 0.69	0.10 <sup>ns</sup>
♂♂ Dh	0	0	0	0	0	0	
♀♀ Dm + Ds	10.73 $\pm$ 3.22	18.43 $\pm$ 5.95	15.50 $\pm$ 4.52	0.04 <sup>ns</sup>	42.38 $\pm$ 11.58	41.34 $\pm$ 10.65	0.05 <sup>ns</sup>
♀♀ Db	0.47 $\pm$ 0.33	0.33 $\pm$ 0.25	0.30 $\pm$ 0.24	0.83 $\pm$ 0.36	0.83 $\pm$ 0.36	0.83 $\pm$ 0.33	0.36 <sup>ns</sup>
♀♀ Dh	0	0	0	0	0	0	
Total	22.47 $\pm$ 6.08	37.30 $\pm$ 11.26	31.27 $\pm$ 9.13	0.21 <sup>ns</sup>	81.55 $\pm$ 21.48	83.24 $\pm$ 21.34	0.34 <sup>ns</sup>

Species <sup>a</sup>	108 h		z
	Control half	Supplemented half	
♂♂ Dm	22.46 $\pm$ 3.88	21.86 $\pm$ 3.56	0.48 <sup>ns</sup>
♂♂ Ds	29.56 $\pm$ 3.67	29.46 $\pm$ 3.77	0.35 <sup>ns</sup>
♂♂ Db	1.35 $\pm$ 0.33	6.78 $\pm$ 1.65	3.39 <sup>***</sup>
♂♂ Dh	0.87 $\pm$ 0.38	1.24 $\pm$ 0.68	0.44 <sup>ns</sup>
♀♀ Dm + Ds	54.67 $\pm$ 7.02	51.19 $\pm$ 6.83	0.74 <sup>ns</sup>
♀♀ Db	1.62 $\pm$ 0.40	6.63 $\pm$ 1.57	3.13 <sup>**</sup>
♀♀ Dh	0.71 $\pm$ 0.40	1.51 $\pm$ 0.84	1.02 <sup>ns</sup>
Total	111.24 $\pm$ 14.13	118.67 $\pm$ 15.21	0.66 <sup>ns</sup>

108 h		z
Control half	Whole fruit	
44.10 $\pm$ 8.1	52.03 $\pm$ 9.24	2.41 $\pm$ 0.71
2.28 $\pm$ 1.31	103.13 $\pm$ 15.98	2.51 $\pm$ 0.76
2.57 $\pm$ 1.44	209.03 $\pm$ 31.28	

<sup>a</sup> Dm = *D melanogaster*, Ds = *D simulans*, Db = *D buzzatii*, Dh = *D hydei*. <sup>b</sup> Wilcoxon matched-pair signed-rank test (Siegel and Castellan, 1988). <sup>ns</sup> Indicates  $P > 0.05$ ; \*\* indicates  $P < 0.01$ ; \*\*\* indicates  $P < 0.001$ .

**Table II.** Mean wing length (mm  $\pm$  SD) of males emerged from control and supplemented halves of 108 h fruits.

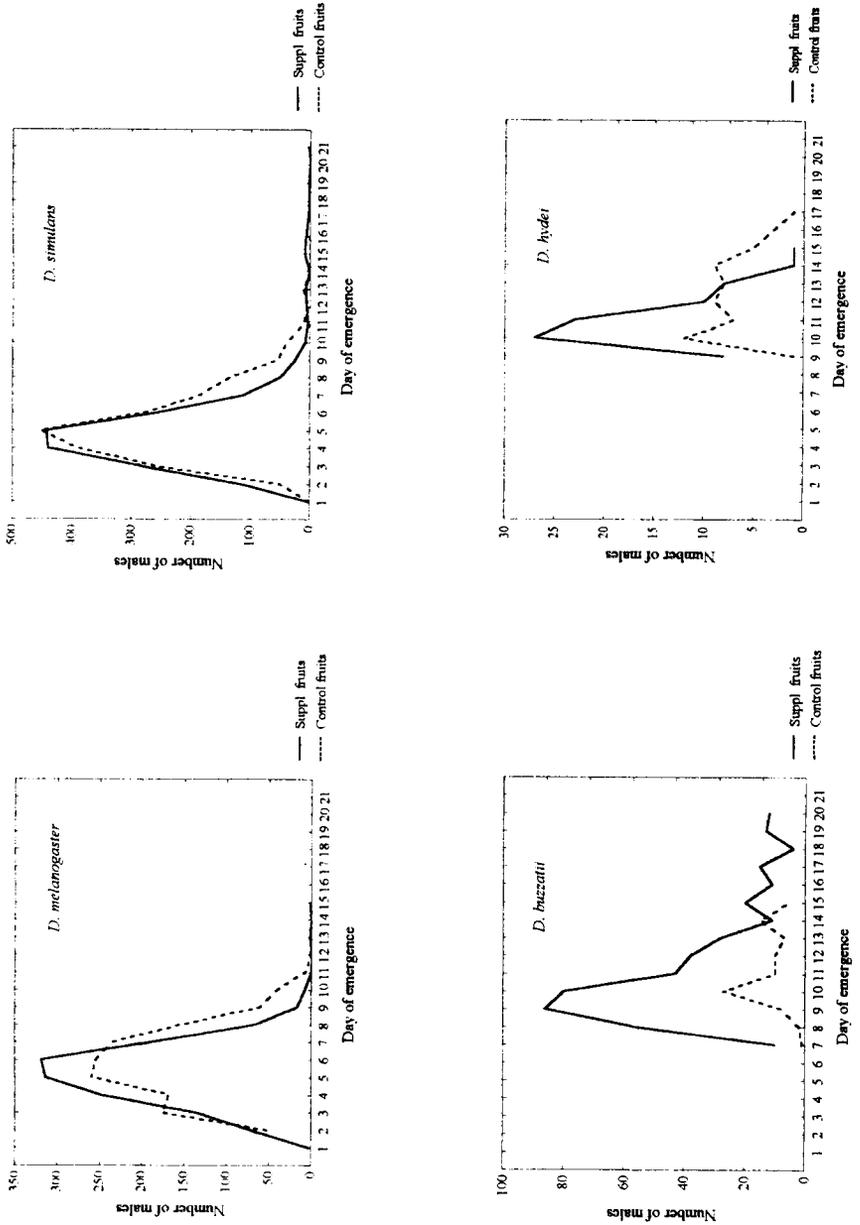
Species	108 h		$t_s^b$	df
	Control half	Supplemented half		
♂♂ Dm	1.901 $\pm$ 0.069	1.905 $\pm$ 0.053	0.65 <sup>ns</sup>	40
♂♂ Ds	1.721 $\pm$ 0.055	1.721 $\pm$ 0.031	0.21 <sup>ns</sup>	50
♂♂ Db	1.863 $\pm$ 0.131	1.951 $\pm$ 0.089	2.92*	13
♂♂ Dh	2.537 $\pm$ 0.131	2.596 $\pm$ 0.062	1.60 <sup>ns</sup>	3

<sup>a</sup> Dm = *D melanogaster*, Ds = *D simulans*, Db = *D buzzatii*, Dh = *D hydei*. <sup>b</sup> Paired *t*-test. <sup>ns</sup> Indicates  $P > 0.05$ ; \* indicates  $P < 0.05$ .

### Analysis of population structure

Table III presents summary  $F$ -statistics for the *D buzzatii* flies that emerged from the 108 h *Opuntia* fruits (the raw data are available upon request to the corresponding author). For selectively neutral loci, the extent of genetic differentiation of the subpopulations in the present situation, where there is only one round of drift and random mating in the population at large (Quezada-Díaz et al, 1992; Barbadilla et al, 1994), is characterized by  $N_e$ , the effective number of locally breeding adults (Wade and McCauley, 1988). All confidence intervals for  $F$  values in the 108 h supplemented fruits included zero, which suggests that  $N_e$  is relatively large (see below). On the other hand,  $F_{IS}$  and  $F_{ST}$  values were different from zero in the 108 h control fruits, indicating that there is an excess of heterozygotes within, and a substantial differentiation among, limited resource fruits. The confidence intervals for  $F_{IS}$  do not overlap, and this could be taken as a real difference between the supplemented and the control fruits. However, some caution is needed with this interpretation because it could be argued that with four loci (as here), and under the null hypothesis  $H_0: F_{IS} = 0$ , estimates of this parameter are negative for all loci with a probability of  $(1/2)^4 = 0.0625$ , and zero will be included in the confidence interval. Raymond and Rousset (1995b) have recently stressed that bootstrap resampling to build a confidence interval is incorrect when the number of loci is small.

Exact tests for population differentiation (Raymond and Rousset, 1995a, b) comparing control and supplemented fruits provided no evidence for allelic heterogeneity at any locus. Analysis of two-locus linkage disequilibrium coefficients showed only eight significant disequilibria out of 179 possible comparisons. Four of these pairs have both members located on the second chromosome, and the other four involved the *Adh-1* locus. Thus, no clear patterns were found, and this result could be taken as another reflection of large effective population size within *Opuntia* fruits (see below).



**Fig 1.** Distribution of emergences through time in 108 h control and supplemented *O ficus-indica* fruits for the four *Drosophila* species.

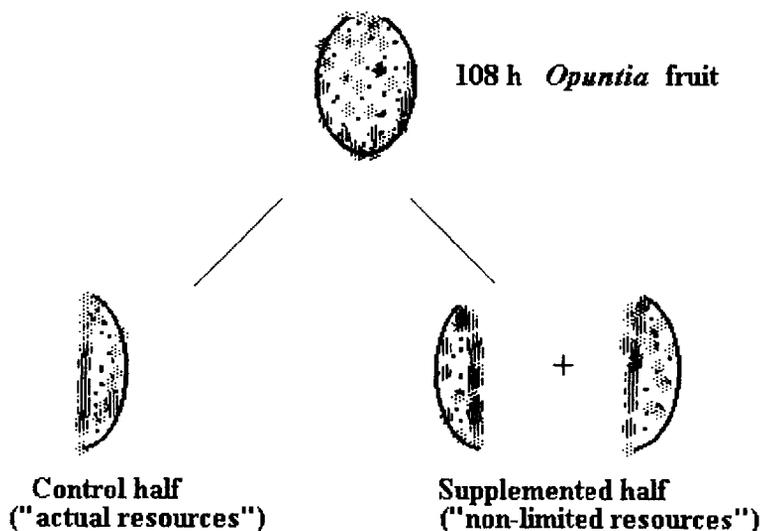
Table III. *F*-statistics on adult *Drosophila buzzatii* flies emerging from *Opuntia ficus-indica* fruits.

<i>Locus</i>	108 h Control halves (n = 12)		108 h Supplemented halves (n = 19)	
	<i>F<sub>IS</sub></i>	<i>F<sub>IT</sub></i>	<i>F<sub>St</sub></i>	<i>F<sub>IS</sub></i>
<i>Est-2</i>	-0.1117	-0.0592	0.0473	0.0409
<i>Aldox</i>	-0.0046	0.0586	0.0629	0.0107
<i>Pept-2</i>	-0.0692	-0.0804	-0.0104	0.0223
<i>Adh-1</i>	-0.1073	-0.0149	0.0834	-0.0371
Mean	-0.0771	-0.0276	0.0460	0.0116
Bootstrap confidence intervals	-0.1099 -0.0309	-0.0681 0.0229	0.0076 0.0735	-0.0229 0.0353
<b>Grouping <i>Est-2</i> alleles</b>				
Mean	-0.0687	-0.0187	0.0468	0.0067
Bootstrap confidence intervals	-0.0992 -0.0270	-0.0637 0.0345	0.0074 0.0755	-0.0229 0.0275
				-0.0149 0.0541
				-0.0006 0.0277

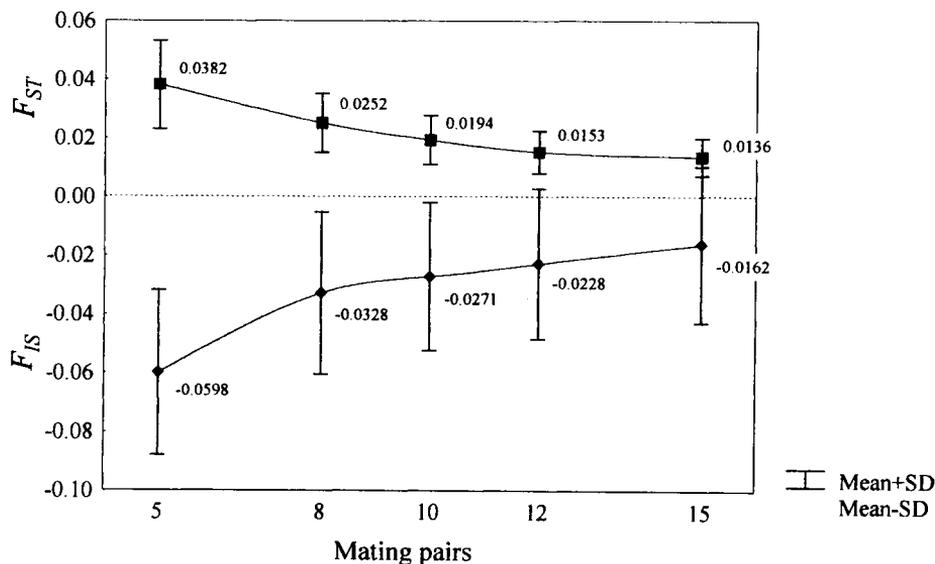
95% confidence limits are from a bootstrap procedure resampling individual fruit contributions 1 000 times.

### *Estimate of the number of parents breeding on a single fruit*

Given that flies from the supplemented halves have developed in 'non-limited resources', it is important to recall that the corresponding  $F$ -values in table III do not mix drift and selection but probably reflect the sampling effect. Therefore, we used computer simulations to compare  $F$ -statistics from supplemented fruits with the expected values generated by various numbers of mating pairs contributing to a breeding site. The main steps of our reasoning for disentangling the effects of drift and selection are summarized in figure 2. Different numbers of mating pairs were selected randomly to contribute to 19 breeding sites, and a number of offspring equal to that obtained for the supplemented fruits (between 5 and 80 individuals per fruit) was sampled at random from each breeding site. Even though theoretical predictions about the expected  $F$ -values could be easily made from the above assumptions (see below), we think it may be useful to have an idea of the standard deviations of their distributions in this particular case (ie, equal number of fruits and emerging adults than in the actual sample).  $F$ -statistics were calculated according to the methods of Weir (1990), and 100 simulations were undertaken for each set of conditions. The interactive matrix algebra program MATLAB (V 4.0 for Windows) was used for computations on a 486 (66 Mhz) PC-compatible. For simplicity, we considered four biallelic loci (alleles other than *Est-2<sup>a</sup>* were grouped into a single class) with allele frequencies in the total population equal to those estimated from the supplemented halves. Estimates of  $F$ -statistics after grouping alleles are also given at the bottom of table III.



**Fig 2.** Experimental procedure and simulation steps for disentangling the effects of drift and selection on  $F$ -statistics. Simulation steps: 1) estimate the number of founders from the supplemented halves; 2) build a confidence interval for  $F_{IS}$  in the control fruits due to 'drift'; 3) estimate the probability of obtaining the actual  $F_{IS}$  (= -0.0687).



**Fig 3.** Expected values for  $F_{ST}$  and  $F_{IS}$  estimates of supplemented fruits when different numbers of mating pairs taken at random from the total population contribute to the progeny of each fruit. Each point represents the mean ( $\pm$ SD) of 100 simulations. The points have been connected by cubic spline interpolation to enhance visibility.

$F_{ST}$  and  $F_{IS}$  values approach zero (fig 3) as more mating pairs contribute to a site, and comparisons with the actual values in table III (ie, those from the supplemented halves) suggest that at least 15 females oviposit on a site. The expected values of  $F_{IS}$  in figure 3 could also be approximated by  $-1/(2N_e - 1)$  (Kimura and Crow, 1963). Thus, for five, ten and 15 mating pairs  $F_{IS}$  approximates to  $-0.053$ ,  $-0.026$  and  $-0.017$ , respectively, which are very close to the numerical values. The simulations, however, assume that females contribute equally to the total number of eggs of each fruit, but the actual estimate is for an effective number of mating pairs.

Let us accept the figure of 15 mating pairs contributing progeny to a site. It would be possible, then, to overcome the difficulties with the bootstrap method and build a confidence interval for the  $F_{IS}$  value in the control fruits under the hypothesis that selection has a negligible effect on the allozyme variation. To do this, we generated 500 independent samples that matched the situation for the control fruits, ie, the mating pairs were selected randomly to contribute to 12 breeding sites, and a number of individuals equal to the actual sample size was taken at random per site. Figure 4 shows the distribution of the  $F_{IS}$  values obtained. We find that the probability of  $F_{IS}$  being less than or equal to  $-0.0687$  (ie, the actual value in the control fruits) is 0.162, and it is clear that we cannot support the hypothesis of a heterozygote excess in the control fruits caused by selection with the evidence from the simulations.

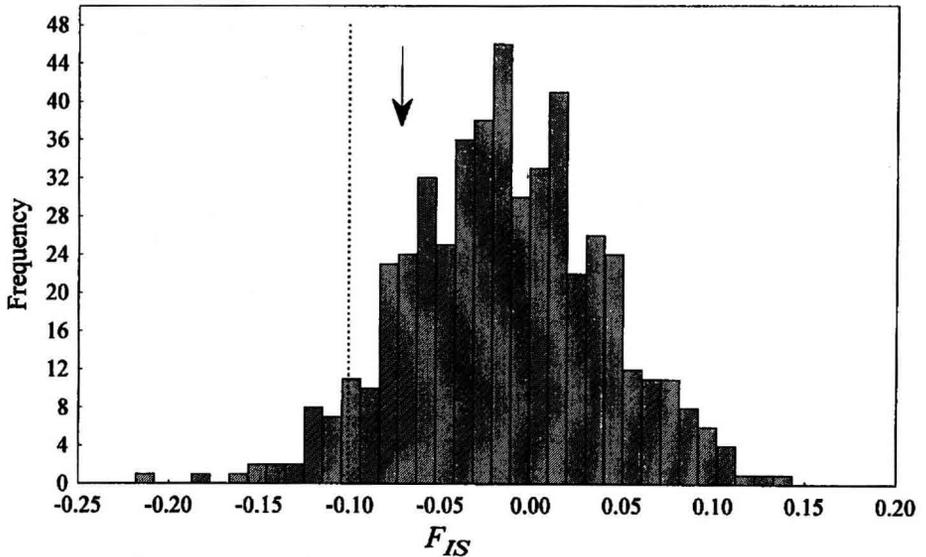


Fig 4. Distribution of 500  $F_{IS}$  estimates obtained from computer simulations that matched the sample sizes in the control fruits. We are assuming that 15 randomly selected mating pairs are contributing to the progeny emerging from a fruit. The one-tailed 5% rejection region is marked off by the vertical dotted line. The arrow identifies the actual estimate being tested ( $-0.0687$ ).

## DISCUSSION

We have shown that the larvae of *D. buzzatii* are resource limited in *Opuntia* fruits, and this species suffers density-dependent mortality in the field (table I). In addition to larval viability, resource limitation also has a significant effect on body size, a trait phenotypically positively correlated with adult fitness components (Santos et al, 1988, 1992; Leibowitz et al, 1995). The relevance of resource structure for expected mating success and/or fertility of the fly is clearly indicated by the substantial differences found in the individual body size due to properties of the breeding sites in which it grew as a larva (Prout and Barker, 1989), and both of these effects can be interpreted as demonstrating that there is a large opportunity for selection under natural conditions. The same conclusion had been previously reached by Grimaldi and Jaenike (1984) for mushroom-feeding *Drosophila*, which suggests that such phenomena have to be considered as general in natural conditions. It should be noted that the fruits in our experiment were only available for oviposition for at most 108 h. If oviposition continues beyond this time, resource limitation in undisturbed *Opuntia* fruits may actually be greater than our data suggest.

When resources are scarce, those species (or individuals within a species) with a short developmental period are presumably at a competitive advantage because larvae are more likely to complete development before their patch is exhausted (Bakker, 1961, 1969; Nunney, 1983; Mueller, 1988; Partridge and Fowler, 1993;

Santos et al, 1994). It is, therefore, interesting that one of the two slow developers in our sample, namely *D buzzatii*, shows a significant effect of additional food. The numbers of *D hydei* flies emerging from the *Opuntia* fruits were too low to obtain meaningful conclusions. Breeding opportunities for *Drosophila* species in *Opuntia* sites are frequent during the fruit season (from August to November at Carboneras), and we could hypothesize that interspecific competition would exclude *D buzzatii* from *Opuntia* fruits because the most common coexisting species, *D simulans*, is at a competitive advantage. However, even though the wing length of *D buzzatii* was significantly negatively correlated with the density of *D simulans* in 108 h control fruits, the relative intensities of intra- and interspecific competition cannot be assessed from our data. Some recent experiments on larval competitive effects measured on semi-natural *O ficus-indica* fruit food at 25 °C suggest that both *D melanogaster* and *D simulans* significantly reduce larval performance of *D buzzatii* (A Galiana, pers comm 1995)

We can think of *Drosophila* population structure as consisting of an array of local breeding populations with high extinction and recolonization rates. The fraction of genetic variance due to the sampling effect of colonization among the newly founded populations is  $F_{ST} = \frac{1}{2 N_e}$ ,  $N_e$  being the effective number of adults breeding on a single patch (Wade and McCauley, 1988). This would be an equilibrium value because the successional changes that take place in the discrete breeding sites are so rapid that there is often time for only a single generation before the patch becomes unusable. Under this simple model, the effect of breeding structure on genetic variation is expected to be uniform over all alleles and loci, whereas natural selection may be expected to act differently on each allele and locus (Lewontin and Krakauer, 1973). Estimates of  $F$ -statistics (table III) suggest that there is an excess of heterozygotes within, and a substantial differentiation among, limited resource fruits when compared with the values obtained from the supplemented halves. This could be taken as evidence for selection acting in some way on the allozyme variation. However, even though the 95% confidence intervals for the  $F_{IS}$  estimates do not overlap, the conclusion that both values are statistically different is not warranted (fig 3). We would like to point out that comparisons of  $F$ -statistics is a somewhat indirect way of detecting selection, and other methods might be more appropriate. The present experimental design could, in theory, allow us to estimate both input (from supplemented halves) and output (from control halves) zygotic ratios in natural breeding substrates. Measures of larval viability could thus be obtained in each site from the cross-product estimator (Manly, 1985), together with confidence intervals. However, the number of *D buzzatii* flies that emerged from the control halves was not large enough to allow meaningful fitness estimates.

In conclusion, our data show that there is density-dependent mortality in the field for *D buzzatii*, and suggest that about 30 individuals contribute progeny to each fruit. The data do not, however, provide an unambiguous answer with respect to the occurrence of selection on allozyme variation.

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