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The evolutionary history of *Drosophila buzzatii.* XXV. Random mating in nature

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Using allozymes as the genetic probe, data are presented which show that wild *Drosophila buzzatii* females and males engaged in copulation mate at random. Hence, putative inbreeding due to local mating of genetically related flies emerging from the patchy distributed substrates, was not detected. We conclude that individuals raised from a niche disperse and mate at random with other members of the population, so only one round of drift due to the colonization of suitable and ephemeral breeding sites is taking place.

Keywords: allozymes, breeding sites, cactophilic *Drosophila*, mating pattern, natural population, population structure.

Introduction

Mating pattern refers to choice of mate and is one of the three main components of mating behaviour besides male and female differential mating success (Lewontin *et al.*, 1968; Anderson & McGuire, 1978; Alvarez & Fontdevila, 1981; Santos *et al.*, 1986). Albeit not a selective component by itself, mating pattern links genotypic frequencies across generations and subsequent gene and gametic frequency changes induced by selection will be dependent on input genotypes. Random mating, which can be loosely defined as 'the absence of any tendency for certain kinds of males and females to pair, when considered with respect to a particular trait' (Falk & Ehrman, 1973), is the simplest pattern of mating and is usually assumed in most population genetic models. Even in some genetic models of sexual selection that involve differential mating success in one sex due to preferences exhibited by the other sex, there are not deviations from random mating (O'Donald, 1980).

Two kinds of departure from random mating have been classically considered by many authors: assortative mating and inbreeding. There is a distinguishing feature between them, i.e. assortative mating with or without differential mating success is character-specific, whereas deviations from random mating due to inbreeding are not specific to particular traits but affect all loci in a uniform way. In nature inbreeding may occur wherever the population is spatially structured. The cactophilic fly *Drosophila buzzatii* feeds and breeds upon the micro-organisms associated with the decaying *Opuntia* (prickly pear) tissues which constitute an ephemeral and patchy resource (Barker, 1977; Santos *et al.*, 1989). The number of parents breeding on a single rot is limited (approximately 10; Santos *et al.*, 1989; Thomas & Barker, 1990), and it has been suggested that inbreeding may be relatively common in *D. buzzatii* and other cactophilic species of the *repleta* group because newly emerged flies from a breeding site, which are more closely related than flies taken at random from the whole population, are more likely to mate with each other (Thomas & Barker, 1990). The purpose of this paper is to test whether or not deviations from random mating are the norm in *D. buzzatii*.

Most tests for the null hypothesis of random mating, or independence of the parents' probabilities of transmitting genes, are based on the fit of observed genotypic frequencies to Hardy–Weinberg proportions. Nevertheless, it is well known that these are indirect tests and cannot bè taken as evidence of random mating because their power to detect departures from Hardy–Weinberg, due to inbreeding (Ward & Sing, 1970) or selection (Lewontin & Cockerham, 1959), is weak (see also Brown, 1970) and, what is more striking, random union of gametes is not equivalent to random mating (Li, 1988; Tai, 1990). Hence, it is necessary to determine the genotypes of the mating

pairs and this can be accomplished either by collecting mother–offspring genotype combinations or by sampling directly both partners. The second procedure can also be used for quantitative traits (Partridge *et al.*, 1987; Santos *et al.*, 1988, 1992). However, both methods are not equivalent as concurrent multiple paternity has been described in many organisms including several *Drosophila* species (Anderson, 1974; Milkman & Zeitler, 1974, Cobbs, 1977; Gromko *et al.*, 1980; Levine *et al.*, 1980; Loukas *et al.*, 1981), and it would be difficult, for autosomal loci, to distinguish between mating by two homozygous or a single heterozygous male. Consequently, sampling mating pairs in nature seems to be the most accurate method and is the one used in this paper.

Materials and methods

The present study was carried out in the early summer of 1987 (25–29 June) and in the late spring of 1989 (16–20 June) in an old *Opuntia ficus-indica* plantation located at Carboneras, which has been described elsewhere (Ruiz *et al.*, 1986). No rotting *O. ficus-indica* fruits were present in the sampling area at the time of collection, so *D. buzzatii* flies were feeding and breeding exclusively on rotting cladodes. Sampling procedures were the same in both years and full details are given in Santos *et al.* (1988, 1992). Briefly, the flies attracted to the daily collected rots were observed and mating pairs were gently aspirated by use of a tube operated by mouth-suction. The number of mating pairs sampled was 396 in 1987 and 386 in 1989. Within the next 3 h after collection the flies were separated by sex and individually mated to virgin flies of a laboratory stock to ascertain their karyotype (Ruiz *et al.*, 1991). After approximately 2 weeks, thorax length measurements were carried out and the flies were frozen at − 20°C for later starch gel electrophoresis.

Each adult was assayed for two polymorphic enzyme loci (*Pept-2* and *Adh-1*) in 1987 and four (*Est-2, Pept-2, Aldox* and *Adh-1*) in 1989. Details of the electrophoretic procedures are given in Loukas & Krimbas (1980) for *Pept-2* and in Barker & Mulley (1976) and Barker *et al.* (1986) for the other three. All four loci are autosomal and linkage studies have shown that *Est-2* and *Aldox* are linked to the inversions on the second chromosome, whereas *Pept-2* is outside the inverted fragments (J. E. Quezada-Diáz *et al.*, unpublished results; see also Knibb *et al.*, 1987 and Thomas & Barker, 1990). *Adh-1* (closely linked to locus *Adh-2*; see Oakeshott *et al.*, 1982 and Alberola *et al.*, 1987) maps at the G1a band of the third chromosome as deduced from the strong signal obtained by in-situ hybridization with a fragment containing the *Adh* gene of *D. melanogaster* (Labrador *et al.*, 1990). In Carboneras, *Est-2* segregates for four alleles, and the other three loci segregate for two alleles each (see Table 1). The nomenclature used for *Est-2, Aldox*, and *Adh-1* alleles is that given in Barker & Mulley (1976) and Barker *et al.* (1986) and was standardized by using *D. buzzatii* stocks kindly sent to us by Professor J. S. F. Barker. The same standard nomenclature was used for *Pept-2*; i.e. *Pept-1* (monomorphic in this population) encoded for a faster migrating protein than *Pept-2*, and *Pept-2*^{a} allele encoded for a faster migrating protein than *Pept-2*^{b}. Alleles *Est-2*^{b}, *Est-2*^{c} and *Est-2*^{d} were pooled into a single class for statistical analyses.

Three-way contingency tables per locus and year were obtained and statistically analysed with respect to the following variables: day of sampling (α), female genotype (β), and male genotype (γ). The data were fit to a series of log-linear models (see Bishop *et al.*, 1975) and a brief description follows.

The saturated log-linear model for a three-dimensional table is:

$$\ln f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk},$$

where f_{ijk} is the expected frequency in row i, column j, depth k of the three-way contingency table, and μ is the mean of the logarithms of the expected frequencies. This model includes the main effect parameters together with first-order ($\alpha\beta_{ij}$, $\alpha\gamma_{ik}$, $\beta\gamma_{jk}$) and second-order ($\alpha\beta\gamma_{ijk}$) effects. A set of hierarchical models from the simplest one (without two-factor and, by implication, three-factor effects) to the above saturated one can be chosen for fitting to the observed data. Our main goal in the present paper is to test for departures from random mating (i.e. to test for the null hypothesis H_0: $\beta\gamma_{jk} = 0$ for all jk), and the following log-linear models were used:

Model 1: $\ln f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k$,
Model 2: $\ln f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \beta\gamma_{jk}$.

The three variables are completely independent in model 1, whereas variable α is independent of the other two, which are associated with each other, in model 2. In our data, agreement with model 2 would mean that both male and female genotype distributions were similar among days, but observed cell frequencies for female–male genotype combinations were different to their expectations based on marginal frequencies, i.e. there was no random mating.

Two other models (3 and 4) can be used to test for heterogeneity of genotypic frequencies among days in females (some $\alpha\beta_{ij} \neq 0$), and males (some $\alpha\gamma_{ik} \neq 0$),

respectively:

Model 3: $\ln f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij}$,
Model 4: $\ln f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\gamma_{ik}$.

To test for the significance of a term in the log-linear model the corresponding G-statistic is computed as the difference between the G-values corresponding to two of the models: one with the term present and one with it left out (Sokal & Rohlf, 1981). Data manipulation and analyses were accomplished on a Vax-8800 VMS at the Centro de Cálculo de la Universidad Autónoma de Barcelona. The statistical programs used were part of the BMDP Statistical Software (1988).

Results

The total number of mating females and males assayed per locus and year, together with allele frequencies and departures from Hardy–Weinberg proportions (D-value or one-locus disequilibria; see Weir, 1990), are given in Table 1. *Pept-2* showed a statistically significant heterozygote deficiency in females in 1989, whereas an excess of heterozygotes was observed for *Est-2* (females and males) and *Aldox* (only males). No significant differences were detected for allelic frequencies between sexes (G-values ranged from 1.88 for *Pept-2* in 1987 to 0.01 for *Adh-1* in 1989) nor between years ($G = 1.26$ for *Adh-1* and $G = 0.02$ for *Pept-2*) in any case. Table 2 shows the raw data for the female–male genotype combination at each locus on each day of sampling. The total numbers of flies in the collapsed mating tables differ from those given in Table 1 because it was not always possible to score both members of each mating pair.

Table 3 gives the G-value for the goodness of fit test for the various models (see Materials and Methods) together with the tests for the corresponding null hypotheses. Among-day genotypic frequencies of females and males were homogeneous for all loci in both years. In 1987, there were no departures from random mating for *Adh-1*, but female–male genotype combinations deviate from marginal expectations for *Pept-2*. Inspection of the mating tables for this locus indicated that significance was mostly due to the fifth day of collection. To test whether the diagonal counts were significantly larger than expected by chance, i.e. to test for positive assortative mating caused by inbreeding or assortment because of a common ancestral relationship, the kappa coefficient (also known as Cohen's measure of agreement; see Bishop *et al.*, 1975; Kotz & Johnson, 1983) for square two-way tables was computed in the female–male collapsed tables. There was neither evidence of assortment for *Adh-1* or *Pept-2*, nor a clear pattern of deviations from random mating in the case of *Pept-2*. We concluded, therefore, that no obvious biological phenomenon was disturbing the expected mating frequency in the population from which samples were taken. In any case, we repeated the study in 1989 and increased the number of loci to four. The same analyses were carried out for each locus and the G-values are given on the right side of Table 3. Note that random zeros occurred in the contingency

Table 1 Allele frequencies of wild mating *D. buzzatii* females and males, together with the deviation value (D) from Hardy–Weinberg proportions (see text for details)

				Allele frequencies				
Year	Locus	Sex	n	a	b	c	d	D†
1987	*Pept-2*	♀	314	0.467	0.533			−0.0043
		♂	346	0.504	0.496			0.0260
	Adh-1	♀	308		0.565	0.435		−0.0140
		♂	312		0.548	0.452		0.0201
1989	*Pept-2*	♀	349	0.471	0.529			0.0300*
		♂	322	0.497	0.503			0.0140
	Adh-1	♀	332		0.533	0.467		0.0200
		♂	298		0.535	0.465		0.0055
	Aldox	♀	324	0.739	0.261			−0.0186
		♂	327	0.711	0.289			−0.0376**
	Est-2	♀	350	0.756	0.181	0.050	0.013	−0.0225*
		♂	333	0.725	0.182	0.074	0.020	−0.0455**

*$P < 0.05$; **$P < 0.001$.
†Alleles *Est-2*^{b}, *Est-2*^{c}, and *Est-2*^{d} were pooled.

Table 2 Frequencies of *D. buzzatii* wild mating pairs sampled at Carboneras. Rows stand for female genotypes (arranged according to faster migrating alleles; e.g. *Pept-2*$^{a/a}$, *Pept-2*$^{a/b}$, and *Pept-2*$^{b/b}$) and columns for male genotypes

	1987						1989											
Day	*Pept-2*			*Adh-1*			*Pept-2*			*Adh-1*			*Aldox*			*Est-2*		
1	5	4	3	2	8	3	1	6	2	5	5	3	8	14	0	12	18	0
	8	10	5	10	10	4	8	11	3	6	5	8	8	8	1	6	7	2
	1	7	3	1	2	2	3	6	3	6	2	0	1	0	0	0	0	0
2	2	3	3	7	4	2	1	10	5	5	5	2	7	15	2	10	17	0
	10	7	8	7	11	4	4	10	6	2	9	7	8	11	1	8	12	1
	2	5	5	0	4	0	2	6	3	1	4	2	0	0	0	2	1	0
3	3	4	2	6	9	2	7	7	6	3	11	4	22	17	2	24	17	2
	12	15	3	10	11	4	12	18	9	10	16	8	12	12	1	14	22	1
	5	9	7	4	4	5	3	10	9	5	8	5	0	7	0	1	3	0
4	4	7	7	4	10	3	1	5	2	5	8	2	8	9	1	18	5	1
	12	18	5	10	18	5	11	8	6	3	8	2	16	7	0	11	9	1
	5	10	2	6	3	2	7	4	3	6	4	2	2	2	0	0	1	0
5	0	4	6	5	6	4	6	6	6	5	10	3	16	19	3	17	22	0
	10	12	5	5	10	6	5	13	7	12	16	7	16	14	1	15	16	1
	2	9	3	2	4	1	7	17	8	2	10	1	0	1	0	3	1	0

Table 3 *G*-values for models 1–4 and goodness of fit tests for two-factor effects

		1987		1989				
Model	d.f.	*Pept-2*	*Adh-1*	*Pept-2*	*Adh-1*	*d.f.*	*Aldox*	*Est-2*
1	36	39.06	27.59	36.74	37.69	36	43.76	36.97
2	32	26.39	26.60	34.49	34.44	28	36.62	33.34
3	28	34.54	22.93	25.55	31.89	26	28.86	29.53
4	28	32.70	25.05	26.31	26.80	28	37.28	27.56
Hypotheses tested								
H_0: $\beta\gamma_{jk}=0$ for all jk	4	12.67*	0.99	2.25	3.24	8	7.14	3.63
H_0: $\alpha\beta_{ij}=0$ for all ij	8	4.52	4.66	11.19	5.80	10	14.90	7.44
H_0: $\alpha\gamma_{ik}=0$ for all ik	8	6.36	2.54	10.43	10.89	8	6.48	9.41
Kappa coefficients†		−0.019	0.014	−0.013	−0.035		−0.073	0.044
(SE)		(0.043)	(0.048)	(0.041)	(0.044)		(0.053)	(0.051)

*$P<0.05$.
†See text for details.

tables for *Est-2* and *Aldox* (Table 2), so degrees of freedom in Table 3 were correspondingly modified (Brown & Fuchs, 1983). No significant *G*-value was detected this time and model 1 (i.e. complete independence of the three variables) seemed to fit the data perfectly well.

Discussion

Multi-locus studies of *D. buzzatii* natural populations in Australia have often shown heterozygote deficiency at enzyme loci and several explanations have been offered for this observation including the Wahlund

effect or population subdivision, null alleles, and inbreeding (Barker & Mulley, 1976; Barker *et al.*, 1986; Sokal *et al.*, 1987; Thomas & Barker, 1990). The pattern that has emerged from these studies is that different loci do not show the same deficiency of heterozygotes, so it has been postulated that selection plays a role in determining genotypic frequencies in addition to inbreeding (Barker *et al.*, 1986). In contrast to these findings, we have not observed a consistent deficiency of heterozygotes but an excess for the two loci known to be associated with the second-chromosome inversions (*Est-2* and *Aldox*). A similar pattern to that observed in Table 1 emerges from samples of non-mating flies aspirated from *Opuntia* rots or collected by net from fermenting banana baits (to be published elsewhere).

The breeding structure of *D. buzzatii* is similar to that found for other drosophilids living upon nutritionally rich (yeast), yet relatively infrequent, substrates. Females possess numerous synchronously developing ovarioles and probably deposit a bunch of eggs (Santos *et al.*, 1992). There are founder effects associated with the colonization of *Opuntia* rots, so the flies that emerge from a single substrate are more closely related than flies taken at random from the whole population (Santos *et al.*, 1989; Thomas & Barker, 1990). Under a neutral model of genetic differentiation, a general deficiency of heterozygotes is not expected unless more than one round of drift takes place; i.e. unless the flies emerging from a rotting cladode tend to stay and to mate rather than to disperse (Santos *et al.*, 1989). Hence, the crucial point is to know whether or not an *Opuntia* rot remains as a suitable feeding and breeding site for two or more generations.

Most of the rots that develop in the late spring and early summer and are quite ephemeral, drying out in 6–8 weeks, as reported by Barker *et al.* (1983). We do not have a quantitative estimation of the period of time a rot remains as a suitable breeding site at Carboneras, but it seems clear that *O. ficus-indica* is a relatively fast-rotting cactus. Variation in pH during the rotting process is swift and is highly correlated with yeast diversity; i.e. the number of yeast species increases with the age of a rot (F. Peris *et al.*, unpublished observations). From field studies at Carboneras it was evident that the age of a rot (as measured by pH) is a fairly good indicator of the age-structure of *D. buzzatii* immature stages. Thus, at pH 7.0–7.5 all rots that had been previously colonized by *D. buzzatii* females mainly host second- and third-instar larvae. At pH 8.0–8.5, tan-coloured pupae can be seen around the cylinders of the dense and highly reticulate vascular system of the *Opuntia* cladodes, with the future imago easily noticeable in many of them. The observed synchronization between larval development and rotting process agrees with the fact that cactophilic *Drosophila* females lay their eggs early in the rotting process (Starmer *et al.*, 1986). However, even if a rotting *Opuntia* cladode were no longer suitable for oviposition of the raised females, it does not necessarily imply that emerged adults would tend to disperse rather than to remain. This follows from the results of laboratory experiments with *D. buzzatii*, which are consistent with the hypothesis that adults are polyphagous on yeasts but have yeast preference for oviposition (Vacek *et al.*, 1985). Although this situation could result in local random mating subpopulations followed by adult dispersion, the results of this paper clearly discard this possibility at least in the population of Carboneras.

The feasibility of a different situation at other times of the year, when environmental conditions and substrate availability are not the same, remains. Nevertheless, correlation coefficients for thorax length of male and female mating pairs were not statistically different from zero either in early summer or in mid fall (Santos *et al.*, 1992). From a sample of newly emerging adults collected in the field, we have estimated that about 35 per cent of the total phenotypic variance for thorax length is accounted for the among-rot mean variation (M. Santos *et al.*, unpublished observations). Therefore, local random mating of flies raised from a rot can be discarded again as it would result in positive assortative mating for body size in the whole population. In summary, from the traits studied there is strong evidence to conclude that under natural conditions *D. buzzatii* flies mate randomly.

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