# Effect of Breeding Structure on Population Genetic Parameters in Drosophila

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Manuscript received April 29, 2003
Accepted for publication October 27, 2003

ABSTRACT

The breeding structure of populations has been neglected in studies of Drosophila, even though Wright and Dobzhansky's pioneering work on the genetics of natural populations was an attempt to tackle what they regarded as an essential factor in evolution. We compared the breeding structure of sympatric populations of *D. melanogaster* and *D. simulans*, two sibling species that are widely used in evolutionary studies. We recorded changes in population density and microsatellite variation patterns for 3 years in a temperate environment of southwestern France. Results were distinctively different in the two species. Maximum population levels in summer and in autumn were similar and fluctuated greatly over years, each species being in turn the most abundant. However, genetic data showed that *D. melanogaster* made up a continuous breeding population in time and space of practically infinite effective size. *D. simulans* was fragmented into isolates with a local effective size of between 50 and 350 individuals. A consequence of this was that, while a local sample provided a reliable estimate of regional genetic variability in *D. melanogaster*, a sample from the same area provided an underestimate of this parameter in *D. simulans*. In practical terms, this means that variations in breeding structure should be accounted for in sampling schemes and in designing evolutionary genetic models. More generally, this suggests the existence of differential reactions to local environments that might contribute to several genomic differences observed between these species.

INTEREST in the genetic structuring of natural populations arose in the early 1930s, after the population genetic syntheses published by Fisher (1930) and Wright (1931) revealed their diverging opinions concerning the effect of population breeding structure on the dynamics of evolution through natural selection. The need to estimate population parameters led Dobzhansky to investigate the genetics of natural populations in *Drosophila pseudoobscura* (Lewontin *et al.* 1981). Using the allelism of lethals, Dobzhansky and Wright (1941) and Wright *et al.* (1942) were able to provide joint estimates for $N_e$ (the effective population size) and $m$ (the migration rate between populations). These studies led to growing interest in population structuring and encouraged Wright to develop *F*-statistics (Wright 1951). Lethal studies were also conducted in *D. melanogaster* (Ives 1945, 1950, 1959). The availability of balancers in this species made it possible to simultaneously investigate the distribution of deleterious effects (see *e.g.*, Mukai and Yamaguchi 1974). However, lethal studies had limitations due to extensive line heterogeneity in mutation rates resulting from transposable elements (Mukai *et al.* 1985; Ives and Band 1986; Keightley and Eyre-Walker 1999). The introduction of allozyme techniques (Hubby and Lewontin 1966) raised new interest in the breeding structure of Drosophila and led to observations, still to be confirmed, showing substantial local structuring (Danieli and Costa 1977; Taylor and Powell 1977). Microsatellite markers now provide a powerful way of detecting microscale structuring in Drosophila populations (Agis and Schlötterer 2001). The microgeographic distribution of Drosophila populations is still poorly understood despite ecological studies in several species (*e.g.*, Begon 1977). In particular, more information in this domain is needed from *D. melanogaster* and *D. simulans*. These species have become major evolutionary genetics models in the last decade and show confusing differences in their patterns of molecular variation, which suggest demographic explanations (*e.g.*, Andolfatto 2001; Begun 2001; Charlesworth 2001). Moreover, captive Drosophila populations have recently been used as experimental models of endangered species (Frankham 1995), thus providing additional reasons to study their breeding structure in nature.

We report a microscale study of population structuring in *D. melanogaster* and *D. simulans* in the Bordeaux vineyard area of southwestern France. The "fruit fly" or "vinegar fly" (Green 2002; the "vintage fly" or "musset" of local vine growers) lives on fermenting fruit, forming dense populations on the rotting leftovers of vine agriculture. These two species are thought to originate in Africa and to have extended their range to Europe with the rise of agriculture in neolithic times (Lachaise *et al.* 1988; Bénassi and Veuille 1995). Southwestern France was planted with vines in the Roman era and has re-

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**TABLE 1**

**Sampling scheme**

| Sample | Sampling site | Species | Sampling time (day/mo/year) | Sample 2*n* |
|---|---|---|---|---|
| Bordeaux 1-96 | Grande Ferrade | *D. melanogaster* | 19/12/1996 | 20 |
| | — | *D. simulans* | 19/12/1996 | 52 |
| Bordeaux 1-97a | — | *D. melanogaster* | 9/8/1997 | 40 |
| | — | *D. simulans* | 13/8/1997 | 36 |
| Bordeaux 1-97b | — | *D. melanogaster* | 25/8/1997 | 40 |
| | — | *D. simulans* | 25/8/1997 | 42 |
| Bordeaux 1-97c | — | *D. melanogaster* | 8/10/1997 | 38 |
| | — | *D. simulans* | 8/10/1997 | 42 |
| Bordeaux 1-98 | — | *D. melanogaster* | 28/9/1998 | 38 |
| | — | *D. simulans* | 28/9/1998 | 38 |
| Bordeaux 2-97 | Couhins | *D. melanogaster* | 22/8/1997 | 38 |
| | — | *D. simulans* | 22/8/1997 | 20 |
| Preignac 1-97 | Preignac 1 | *D. melanogaster* | 23/8/1997 | 38 |
| | — | *D. simulans* | 23/8/1997 | 40 |
| Preignac 1-98 | — | *D. melanogaster* | 10/9/1998 | 42 |
| | — | *D. simulans* | 8,15/10/1998 | 44 |
| Preignac 2-97 | Preignac 2 | *D. melanogaster* | 23/8/1997 | 36 |
| | — | *D. simulans* | 27/8/1997 | 38 |

mained an active wine-producing region ever since. It was already a wine-exporting province in the middle ages and we can suppose that the region has sustained a Drosophila population for centuries, a period spanning from 5000 to 10,000 Drosophila generations. American populations of these species are thought to have colonized the United States as recently as the 1870s (Sturtevant 1920). The Bordeaux area is evenly and densely planted with vine, thus providing a continuous landscape that is ideal for studying microscale migration. The ecophysiology of fruit flies is poorly understood. In temperate countries natural populations are found only from late August to early December with fluctuations depending on the year. They are rarely found outside of this period. Low density in winter could be critical in determining their effective population size. In this study, we used microsatellite genetic variation to determine changes in effective population size over time and space. Given the paucity of reference literature on the subject, we designed an observational scheme that balanced sampling effort over time and over space, each factor being considered at two nested scales (from 3 months to 3 years and from 4 to 30 km, respectively) to be able to adjust the focus in further studies.

## MATERIALS AND METHODS

**Study area:** The sampling design is summarized in Table 1. It involves four collecting sites that formed two 30-km-distant regions, each being divided in turn into two 4-km-distant sampling sites. The first region is in the Graves vineyard, immediately south of Bordeaux. The two sampling sites were at the "Grande Ferrade" château (hereafter "Bordeaux 1"), an agricultural research station owned by Institut National de la Recherche Agronomique, and at Couhins village (hereafter "Bordeaux 2"). The second region was in the Sauternes vineyard in Preignac, a village that lies on the left bank of the Garonne river, between Cadillac and Langon. Our sampling sites were "Preignac 1" and "Preignac 2." All sampling took place in vineyards and was repeated over 3 years according to the following scheme:

Bordeaux 1: a test sample was made in late autumn 1996 (19 December). Several samples were collected in summer 1997 over an extended period (see below) and in early autumn 1998 (28 September).
Bordeaux 2: samples were collected in summer 1997 (22 August).
Preignac 1: samples were collected in summer 1997 (23 August) and in autumn 1998 (10 September–15 October).
Preignac 2: samples were collected in summer 1997 (23–27 August) for the genetic analysis and also in summer 1998 for the demographic study (see below).

**Long-term study:** In 1997 and 1998, Bordeaux 1, Preignac 1, and Preignac 2 samples were collected twice a week from the first week of August to the first week of November for an ecological survey that will be published elsewhere. In this article, pooled data from these three locations were used for estimating sex-ratio values and species abundance.

**Short-term study in Bordeaux 1:** In Bordeaux 1 samples were taken at several times for the same population in 1997. They were collected between 9 and 13 August (about the time of year when fruit flies are first observed), on 25 August (about the time of peak abundance), and on 8 October (about the time of declining populations).

**Trapping device:** A technical difficulty in the ecological genetics of Drosophila is that, to our knowledge, previous studies trapped flies using attractive baits made of fermenting fruit. Although very efficient, this technique can introduce sampling biases (*e.g.*, Wahlund effects) by attracting flies from a wide area. We therefore used a nonattractive device adapted from traps previously used for collecting aphids (Labonne *et al.* 1983). Each trap consisted of a wooden frame inside which

**TABLE 2**

**Composition of populations**

| Sampling site | *N* females | *N* males | | |
|---|---|---|---|---|
| | | *D. melanogaster* | *D. simulans* | % *D. simulans* |
| Bordeaux 1-1997 | 12,617 | 10,383 | 2,290 | 18.0 |
| Bordeaux 1-1998 | 6,900[a] | 1,673 | 6,264 | 78.9 |
| Preignac 1-1997 | 2,689[a] | 1,821 | 198 | 9.8 |
| Preignac 2-1997 | 7,636[a] | 4,643 | 303 | 6.1 |
| Preignac 1-1998 | 459[a] | 467 | 86 | 15.5 |
| Preignac 2-1998 | 85[a] | 144 | 106 | 42.4 |

[a] Significant deviation from a balanced sex ratio, $P(\chi^2) < 0.05$.

a 25 × 25-cm screen was made using parallel nylon threads spaced at 5-mm intervals. The threads were covered with glue. These traps were hung in the vineyard for 3–4 days. We consider that this method was nonattractive and collected a constant proportion of the flies that went through the screens. There was no indication of saturation or of variation in efficiency over time, although we did not monitor the screens for this. The trapped flies were recovered by covering the traps with turpentine for 30–120 min. The flies were then stored in 70% ethanol/30% TE until DNA extraction. This protocol provided suitable material for PCR amplification. Variations in the output of amplification on a sample-by-sample basis, however, point to unidentified drawbacks of the collecting method, possibly due to decay of trapped material between field visits. There were 5–15 trapping screens at 1.5-m intervals/sampling site. For each of them, an individual sample of 20 males was used for analysis. Screens from the same area in the same vineyard were pooled when necessary; however, this produced no artifactual structuring (see RESULTS). The number of chromosomes observed is indicated as $2n$ in Table 1.

**Recording genetical data:** DNA extraction, PCR amplification, and examination of polymorphism at 10 microsatellite loci from chromosome II [*bib*, *cad*, *dl*, *Elf-1*, *mam*, *odd*, *slobo*, *Su(H)*, *Su(z)2*, and *twist*] were carried out as described by MICHALAKIS and VEUILLE (1996). These loci are taken from long coding trinucleotide repeats that vary widely in the number of codons within species, but are relatively constant in the average length across species (MICHALAKIS and VEUILLE 1996; COBB *et al.* 2000; VEUILLE *et al.* 2004). These repeated regions are frequent in Drosophila regulatory proteins associated with development and neurogenesis, and normalizing selection seems negligible in terms of deviation from a neutral distribution at the population scale (MICHALAKIS and VEUILLE 1996). When used as genetic markers in different species, these loci do not appear to be liable to ascertainment bias, contrary to noncoding microsatellites (MOUSSET and DEROME 2004; VEUILLE *et al.* 2004). The 10 loci used are evenly spaced over chromosome II, which makes up 40% of the Drosophila genome.

We used only males, since the females of these two species are morphologically similar. Standard statistical analyses were carried out using Genepop version 3.3 of March 2001 (RAYMOND and ROUSSET 1995). This program carries out a Hardy-Weinberg exact test using a Markov chain method from GUO and THOMPSON (1992). It also tests deviation at several loci simultaneously using ROUSSET and RAYMOND's (1995) method. We could use this option since the markers are evenly spaced on the Drosophila genetic map and can be assumed to be independent, except for those linked to the breakpoints of the *In(2L)t* inversion in *D. melanogaster* (MICHALAKIS and VEUILLE 1996). Population differentiation was tested using an exact test (RAYMOND and ROUSSET 1995). Wright's $F_{ST}$ was calculated after WEIR and COCKERHAM (1984). Population heterozygosity was estimated as

$$\hat{H} = 2n/(2n - 1)(1 - \sum p_i^2),$$

where $p_i$ is the frequency of the $i$th allele in a sample of $2n$ chromosomes. The estimate of effective population size and of its confidence interval (C.I.) was calculated using WAPLES's (1989) "*F*" genetic correlation coefficient between samples taken from the same population at different generations. It is estimated as

$$\hat{F} = (1/k)\sum[(x_i - x_j)^2]/[(x_i + x_j)/2 - x_i x_j],$$

where $x_i$ and $x_j$ are allele frequencies in successive population samples, the sum being calculated over $k$ alleles. According to a classical relation, genetic variation decreases as $H_t = H_0 \exp(-t/2N_e)$ between generations 0 and $t$ (WRIGHT 1931; MALÉCOT 1946, 1948). From this, WAPLES (1989) showed that $F$ depends on the effective population size and on sample sizes ($2n_1$ and $2n_2$) according to $E(F) \approx (2n_1 + 2n_2)/(8n_1n_2) + t/(2N_e)$, from which the effective population size can be obtained. Confidence intervals ($\alpha = 0.05$, $1 - \alpha = 0.95$) were computed according to WAPLES (1989) as $\lfloor n\hat{F}/(\chi^2_{(\alpha)/2n}),\ n\hat{F}/(\chi^2_{(1-\alpha)/2n})\rfloor$. Significance levels in multiple tests of genetic differentiation between samples were determined according to the sequential Bonferroni method, using the total number of tests as a reference. It was therefore slightly conservative in pairwise comparisons, since the tests were not independent.

## RESULTS

**Population density levels:** Table 2 shows the total number of male flies collected in Bordeaux 1, Preignac 1, and Preignac 2 over 3 months (August–October) in 1997 and 1998. There were large fluctuations, mostly due to differences in maximal values over years and over locations (E. GRAVOT, unpublished results). The dominant species was *D. melanogaster* in all Preignac samples. In Bordeaux 1, the dominant species changed from 82.0% *D. melanogaster* in 1997 to 78.9% *D. simulans* in 1998. We do not know whether the distribution was the same in females. In pooled data for the two species, the sex ratio fluctuated over time, a balanced sex ratio

**TABLE 3**

**Gene diversity for 10 loci from chromosome II in *D. melanogaster***

| Population | *bib* | *cad* | *dl* | *Elf-1* | *mam* | *odd* | *slobo* | *Su(h)* | *Su(z)2* | *twi* | Average[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bordeaux 1-96 | 0.647 | 0.660 | md | md | md | md | 0.366 | md | 0.797 | 0.111 | 0.516 |
| Bordeaux 1-97a | 0.622 | 0.768 | 0.232 | 0.391 | 0.572 | 0.055 | 0.545 | 0.097 | 0.646 | 0.000 | 0.393 |
| Bordeaux 1-97b | 0.718 | 0.710 | 0.310 | 0.099 | 0.330 | 0.000 | 0.530 | 0.050 | 0.579 | 0.050 | 0.338 |
| Bordeaux 1-97c | 0.690 | 0.679 | 0.373 | 0.280 | 0.509 | 0.000 | 0.511 | 0.160 | 0.538 | 0.053 | 0.379 |
| Bordeaux 1-98 | 0.727 | 0.616 | 0.538 | 0.199 | 0.421 | 0.000 | 0.434 | 0.292 | 0.617 | 0.053 | 0.390 |
| Bordeaux 2-97 | 0.713 | 0.652 | 0.319 | 0.251 | 0.497 | 0.000 | 0.431 | 0.184 | 0.638 | 0.094 | 0.378[b] |
| Preignac 1-97 | 0.697 | 0.675 | 0.464 | 0.347 | 0.519 | 0.059 | 0.458 | 0.191 | 0.560 | 0.000 | 0.397 |
| Preignac 1-98 | 0.671 | 0.693 | 0.360 | 0.422 | 0.628 | 0.138 | 0.417 | 0.000 | 0.496 | 0.000 | 0.383 |
| Preignac 2-97 | 0.666 | 0.578 | 0.598 | 0.317 | 0.348 | 0.000 | 0.511 | 0.104 | 0.616 | 0.000 | 0.374 |

md, missing data.
[a] Average heterozygosity over 10 loci.
[b] Significant excess of homozygotes.

being found only once in six observations. In these three populations, trapped males were significantly less abundant than trapped females in August and September and reached a 50% proportion in October (data not shown). The cause of these variations is unknown. They may thus result from differences in the primary sex ratio, from differential survival, from differential migration, or from differential activity, in which case they would depend on the trapping device.

**Genetic variation levels:** Heterozygosity levels are shown in Table 3 (*D. melanogaster*) and Table 4 (*D. simulans*). Due to technical difficulties and to a small sample size, genetic variation for Bordeaux 1-96 in *D. melanogaster* was calculated from only 5 loci: *bib*, *cad*, *slobo*, *Su(z)2*, and *twist*. Data for all other samples were calculated using 10 loci and were very similar. All loci were polymorphic in *D. melanogaster*. Only 8 loci were polymorphic in *D. simulans*. However, the 2 monomorphic loci in the latter species (*odd* and *twi*) also showed low variation levels in *D. melanogaster*. Overall, *D. melanogaster* was less variable than *D. simulans*. The average heterozygosity was 0.375 in the first species (range 0.338–0.397 over 10 loci) and 0.410 in the second species (range 0.363–0.482 over 10 loci). Three *D. simulans* samples showed especially low values (Bordeaux 1-98, Preignac 1-97, and Preignac 2-97). Two of these samples correspond to sites that were sampled for two successive years. The Bordeaux 1-98 sample showed a marked decrease in heterozygosity relative to the preceding sample (Bordeaux 1-97) since the average value between samples dropped from 0.454 to 0.363. Preignac 1-97 showed a much lower value than did the following sample, Preignac 1-98, with an increase from 0.374 to 0.433. The Preignac 2 sample was studied only once. Interestingly, two of these three low-variation samples (Preignac 1 and Preignac 2) were taken the same year (1997) from neighboring sites. An inspection of genetic diversity at individual loci reveals similar tendencies. In the two Preignac samples, *mam*, *slobo*, and *Su(H)* showed a marked decrease in variability, leading to their lowest values for the whole survey. The values at the other loci were also very similar in the two samples. The third low-variation sample (Bordeaux 1-98) showed a decrease in heterozygosity for a different set of loci: *bib*, *dl*, *slobo*,

**TABLE 4**

**Gene diversity for 10 loci from chromosome II in *D. simulans***

| Population | *bib* | *cad* | *dl* | *Elf-1* | *mam* | *odd* | *slobo* | *Su(h)* | *Su(z)2* | *twi* | Average[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bordeaux 1-96 | 0.684 | 0.268 | 0.707 | 0.505 | 0.438 | 0.000 | 0.576 | 0.590 | 0.637 | 0.000 | 0.441 |
| Bordeaux 1-97a | 0.749 | 0.252 | 0.688 | 0.246 | 0.358 | 0.000 | 0.646 | 0.572 | 0.693 | 0.000 | 0.420 |
| Bordeaux 1-97b | 0.760 | 0.361 | 0.728 | 0.444 | 0.655[b] | 0.000 | 0.566 | 0.577 | 0.732 | 0.000 | 0.482 |
| Bordeaux 1-97c | 0.773 | 0.309 | 0.709 | 0.390 | 0.485 | 0.000 | 0.738 | 0.497 | 0.715 | 0.000 | 0.462 |
| Bordeaux 1-98 | 0.738 | 0.368 | 0.615 | 0.390 | 0.501 | 0.000 | 0.152 | 0.219 | 0.647 | 0.000 | 0.363 |
| Bordeaux 2-97 | 0.810 | 0.363 | 0.604 | 0.209 | 0.600 | 0.000 | 0.758 | 0.608 | 0.736 | 0.000 | 0.469 |
| Preignac 1-97 | 0.803 | 0.371 | 0.757 | 0.483 | 0.142 | 0.000 | 0.549 | 0.000 | 0.637 | 0.000 | 0.374 |
| Preignac 1-98 | 0.736 | 0.342 | 0.677 | 0.347 | 0.467 | 0.000 | 0.596 | 0.507 | 0.656 | 0.000 | 0.433[b] |
| Preignac 2-97 | 0.680 | 0.397 | 0.686 | 0.398 | 0.219[b] | 0.000 | 0.544 | 0.351 | 0.508 | 0.000 | 0.378 |

[a] Average heterozygosity over 10 loci.
[b] Significant excess of homozygotes.

**TABLE 5**

**Genetic differentiation (Weir and Cockerham's $F_{ST}$) among samples of *D. melanogaster***

| Samples | Bx-1-96 | Bx-1-97a | Bx-1-97b | Bx-1-97c | Bx-1-98 | Bx-2-97 | Pr-1-97 | Pr-1-98 |
|---|---|---|---|---|---|---|---|---|
| Bordeaux 1-97a | −0.014 | | | | | | | |
| Bordeaux 1-97b | 0.007 | 0.000 | | | | | | |
| Bordeaux 1-97c | −0.002 | −0.014 | −0.002 | | | | | |
| Bordeaux 1-98 | 0.009 | 0.021 | 0.017 | 0.005 | | | | |
| Bordeaux 2-97 | −0.021 | −0.005 | 0.001 | −0.010 | −0.003 | | | |
| Preignac 1-97 | −0.005 | −0.009 | 0.001 | −0.013 | −0.011 | −0.002 | | |
| Preignac 1-98 | −0.013 | −0.005 | 0.031 | −0.001 | 0.026 | −0.017 | 0.000 | |
| Preignac 2-97 | −0.001 | 0.012 | 0.006 | 0.001 | 0.006 | 0.029 | −0.001 | −0.004 |

No difference was significant at the 0.05 level using an exact test and the sequential Bonferroni procedure. Bx, Bordeaux; Pr, Preignac.

and *Su(H)*. There is thus no indication that selection at one locus is responsible for the dramatic decrease in frequency in three samples, these observations being rather compatible with drift. It also appears that the three outlying samples in *D. simulans* reduce to two variation reduction events, one of these events extending over the 4 km between the two Preignac sampling sites. The general picture is that for most of the time *D. simulans* showed high levels of heterozygosity (range 0.410–0.482) but in three instances dropped to values (0.363–0.378) close to those observed in *D. melanogaster* (0.338–0.397).

The proportion of heterozygotes was not significantly different from values predicted from heterozygosity (*i.e.*, parametric gene diversity) at the population scale, except in two instances, one in each species. This indicates that in general there was no microgeographic structuring within sampling sites. This means that the nonattractive traps used for collecting the flies produced no Wahlund effect or that they recruited flies from a panmictic population that was large enough for randomizing genetic correlation across kin groups. This also indicates that the flies collected at the beginning of the annual demographic expansion showed no consanguinity, contrary to some previous studies (see DISCUSSION).

**Genetic differentiation between samples:** Genetic differentiation between samples involved 36 comparisons. Results are summarized in Table 5 (*D. melanogaster*) and Table 6 (*D. simulans*). None of them was significant in *D. melanogaster*. Sixteen of them were significant in *D. simulans* using the sequential Bonferroni procedure. This is unlikely to result from a higher power of the tests due to the higher heterozygosity in *D. simulans*. Roughly one-half of $F_{ST}$'s in *D. melanogaster* were "negative" (20 *vs.* 16) as expected from sampling fluctuations around a null expectation, compared to very few of them (5 *vs.* 31) in *D. simulans*. These tests are not independent from each other; however, there is no indication that a difference of power of the test in one species is responsible for the contrast found between them. On the contrary, and interestingly, almost all significant tests in *D. simulans* implicate the three samples in which heterozygosity dropped to low values.

No significant differences were observed in either species between the three samples from the time series for Bordeaux 1 in 1997. This indicates that no change in allele frequency occurred during the annual demographic expansion. These samples were therefore pooled. Bordeaux 1-97 then remained significantly different from Bordeaux 1-98 and from Preignac 1-97 in *D. sim-*

**TABLE 6**

**Genetic differentiation (Weir and Cockerham's $F_{ST}$) among samples of *D. simulans***

| Samples | Bx-1-96 | Bx-1-97a | Bx-1-97b | Bx-1-97c | Bx-1-98 | Bx-2-97 | Pr-1-97 | Pr-1-98 |
|---|---|---|---|---|---|---|---|---|
| Bordeaux 1-97a | 0.006 | | | | | | | |
| Bordeaux 1-97b | 0.002 | 0.002 | | | | | | |
| Bordeaux 1-97c | 0.005 | −0.008 | −0.005 | | | | | |
| Bordeaux 1-98 | 0.059[a] | 0.039[a] | 0.036[a] | 0.053[a] | | | | |
| Bordeaux 2-97 | 0.023 | −0.004 | 0.002 | −0.000 | 0.058[a] | | | |
| Preignac 1-97 | 0.068[a] | 0.047[a] | 0.058[a] | 0.044[a] | 0.048[a] | 0.091[a] | | |
| Preignac 1-98 | 0.022[a] | 0.000 | 0.007 | 0.012 | 0.011 | −0.003 | 0.038[a] | |
| Preignac 2-97 | 0.034[a] | 0.003 | 0.025[a] | 0.021 | 0.027 | 0.044[a] | 0.021 | 0.012 |

Bx, Bordeaux; Pr, Preignac.

[a] Significant at the 0.05 level using an exact test and the sequential Bonferroni procedure.

*ulans* (sequential Bonferroni method over 21 comparisons). In Table 6, structuring can be examined between samples from different populations of this species collected at the same time or between samples from the same population collected at different times.

Spatial structuring involved six independent comparisons between the four populations in 1997 (Bordeaux 1, Bordeaux 2, Preignac 1, and Preignac 2) and a unique comparison between two populations in 1998 (Bordeaux 1 and Preignac 1), making a total of seven comparisons. Three of them were significant, all involving 30-km-distant sites in 1997: Bordeaux 1-Preignac 1, Bordeaux 2-Preignac 1, and Bordeaux 2-Preignac 2.

Time structuring involved three pairwise comparisons: the two pairwise comparisons between the three successive Bordeaux 1 samples (1996–1997 and 1997–1998) and those between the two successive Preignac 1 samples (1997 and 1998). Two of them were significant (Table 6): Bordeaux 1 and Preignac 1, when compared between 1997 and 1998.

These results are compatible with the hypothesis that structuring in *D. simulans* results from the low variation observed in Bordeaux in 1998 and in Preignac in 1997. It would thus be a temporary phenomenon.

Since neither the different samples from the Bordeaux area nor those from the Preignac area differed in each species in 1997, samples were pooled within each area to assess the level of genetic differentiation from a larger data set. Genetic differentiation between Bordeaux and Preignac then remained low in *D. melanogaster* (Weir and Cockerham's $F_{ST} = 0.0005$, $P$ value = 0.156) and was somewhat higher in *D. simulans* ($F_{ST} = 0.0302$, $P$ value $< 10^{-5}$).

**Genetic drift in *D. simulans* populations:** Overall these data suggest that *D. simulans* samples were collected in a neighborhood of a small effective size. Genetic differentiation within species can result from either genetic drift alone or a balance between migration and genetic drift. Our sampling design was too simple for us to test detailed models of breeding structure. One way to interpret our data is to consider that the changes are temporal and mainly involve genetic drift. Estimates for $N_e/t$ for a year using Waples's (1989) model under this assumption are: $N_e/t = 352.69$ for Bordeaux 1996–1997 (C.I.$_{0.05}$ = 226.51–633.81); $N_e/t = 48.00$ for Bordeaux 1 1997–1998 (C.I.$_{0.05}$ = 39.61–57.77); and $N_e/t = 99.53$ for Preignac 1997–1998 (C.I.$_{0.05}$ = 76.77–130.02). For Preignac, we assume that the higher heterozygosity in the second year (1998) results from a return to a normal value after a population drop in 1997. There are probably many generations per year. However, in summer and autumn, there are huge fruit-fly populations: the sampling effect of successive generations would not be apparent in a survey of 10 loci on 40 chromosomes. The very low population level in winter suggests that our estimate is close to that of an overwintering generation.

## DISCUSSION

Few studies on the population structure of species from the *melanogaster* subgroup have been carried out. Our observations were conducted at a relatively high latitude (45° N 35′–45° N 45′) and a mild climate in a continuous agrosystem that seems to sustain dense populations of these species. We observed facts that apparently pertain more to demography than to spatial differentiation. While spatial structuring is easy to account for in population genetic studies, demographic perturbations are relatively unpredictible and may differ from place to place, thus confounding genetic analysis. Below, we discuss these facts, discuss their biological meaning, and then consider their consequences for genetical research.

**Microscale contrast between *D. melanogaster* and *D. simulans*:** We found no substantial deviation from Hardy-Weinberg proportions in either species over the period during which these flies are abundant enough to be observed (August–December). This contradicts previous results by Danieli and Costa (1977). These authors recorded EST-6 allozyme variation in six different places from Venetia (Italy) over 2 years, 1971 and 1974. They consistently found an excess of homozygotes at the beginning of the sampling season. Inbreeding decreased in the following generations. They interpreted this as meaning that in late August *D. melanogaster* is composed of micropopulations that have been isolated in winter and have undergone inbreeding. According to their Figure 1, Danieli and Costa (1977) found an inbreeding coefficient $F = 0.6$ in early September, and this value decreased to zero in late October. This is a very high value for an inbreeding coefficient. An average $F = 0.59$ is expected only after four generations of brother-sister mating. No *D. melanogaster* populations are observed for ~7 months, from January to August. *D. melanogaster*'s developmental time in winter must be very long, since it depends on environmental temperature. Development lasts 2 weeks at 25°, but drops to 4 weeks at 17°, a temperature below which males are unable to reproduce. Four generations thus appear a probable maximum for winter populations. The high inbreeding estimate reported by Danieli and Costa (1977) would mean that, for a considerable period of the year, fruit flies are virtually reduced to populations of a couple of breeding individuals. However, our data overlapped the same sampling season, and no evidence of consanguinity was found. The cause of the differences between the two studies is unknown. They differ in a number of aspects: the sampling device, the sampling area, the kind of markers used, and the number of loci. Only comparative studies using the same methods in the two areas could solve this point.

In *D. melanogaster* from southwestern France, no population structuring was found over space or over time. This species thus appears to form a relatively evenly distributed

panmictic population at this geographical scale. For similar latitudes, high population levels (>10,000) were obtained in Japan (Mukai and Yamaguchi 1974) and in the population of Raleigh, North Carolina (Mukai *et al.* 1971; Mukai and Voelker 1997) using the allelism of lethals, with no indication of population substructuring. An allozyme study by Smith *et al.* (1978) found no evidence of microscale structuring in North American *D. melanogaster* populations.

The picture is different in *D. simulans*. Statistically significant differences in allele frequencies were consistently found between locations and between successive years. It is unlikely that significance was due to local heterogeneity within vineyards, since this would also have induced differentiation between sampling sites 4 km apart. Spatial heterogeneity was associated with a reduction in heterozygosity between successive years. In the only case in which this could be observed, the drop in variation was simultaneous 4 km apart, thus giving the spatial extent of a neigborhood. A 4-km patch is substantial, and the effective size relatively small (down to ~50 individuals) for an organism occurring in huge populations in summer.

**Evidence of demographic instability in *D. simulans*:** Overall, the genetic variation in *D. simulans* is much higher than that in *D. melanogaster*, showing that long-term population size is substantial. The significant fixation indices between samples indicate fluctuations in population size in this species. For this reason, we do not interpret the significant $F_{ST}$ values in *D. simulans* as reflecting stable geographic differentiation at a 30-km scale, but as temporary, probably annual, local changes in effective population size. No strong contrast was apparent in population abundance between *D. melanogaster* and *D. simulans*. Depending on the year, either of the two species was the most frequent. This suggests that the difference in effective population size was not due to demographical differences in summer, but probably to differences in winter. It is reasonable to assume that winter populations of either species are fragmented into small overwintering isolates that expand locally in summer, coalesce, and finally restore a dense and continuous population. The most likely population regime would involve two steps, with random genetic drift occurring in winter and gene flow in summer. We can thus imagine the *D. simulans* population as a field of neighborhoods. Fruit flies from a given area would originate from a limited number of surviving individuals, resulting in a temporary level of inbreeding. However, the resulting structuring would not last long, since populations exchange individuals. Our lowest estimates of effective population size in *D. simulans* in Bordeaux is $N_e/t = 48$ for a 1-year cycle. Since the effective population size of a population over some period of time is the geometric mean of the elementary population sizes of each generation, our estimates are likely to be close to the size of the winter breeding population. For the rest of the year, the fruit flies from a given area would retain a gene identity of $f = 1/(2N_e + 1) \sim 1/100$, despite their high population level. It would be tempting to estimate a migration rate between sampling sites. Unfortunately, its estimation would depend on a stable population model, which is confounded by our results. The observation that the decreased heterozygosity in Preignac in 1997 was completely restored in 1998 (and the $F_{ST}$ nonsignificant) suggests that extensive gene flow occurs.

For *D. melanogaster*, no genetic differentiation was detected in this study, but we cannot exclude that fluctuations also exist. We can note only that no bottleneck event was detected in *D. melanogaster* while two strong events were found in *D. simulans* in the same sampling sites. Thus there is a difference, but maybe only one of intensity. Even though the difference between the two species may lie in trivial quantitative changes within a single ecological framework, there are conspicuous differences in the genetic distribution patterns.

We do not assume that the difference observed in southwestern France will be found throughout the overlap zone of these two species, but only that they are able to react differently to their environments in such a location. Microhabitat studies show that *D. melanogaster* is more abundant than *D. simulans* in villages and in houses. This has been observed in a number of areas, including tropical Africa (David 1979) and Tunisia (Rouault and David 1982). This ecological difference is likely to restrict *D. simulans* to unsheltered microhabitats. Moreover, ecophysiological studies carried out by Boulétreau-Merle (1992) on reproduction in Drosophila from southeastern France showed that *D. simulans* is less adapted to temperate areas than is *D. melanogaster*. Temperate *D. melanogaster* females differ from tropical populations in the control of fecundity, thus allowing them to cope with annual environmental changes through individual adaptation. This is not observed in *D. simulans*, where tropical and temperate females behave similarly in experimental designs simulating the two environments (Boulétreau-Merle 1992). These ecological differences would contribute to limit population level in *D. simulans*. A study of 15 *D. melanogaster* populations over a 700-km north-south gradient in southeastern France by Girard and Palabost (1976) showed no allozyme frequency differences. However, groups of populations differed significantly in ovariole number, suggesting ecological adaptations. Unfortunately, no corresponding study is available for *D. simulans*.

**Practical consequences for population genetic studies:** Of the two species used in this study, *D. melanogaster* appears to form a large and stable population, whereas *D. simulans* appears to be fragmented into small drifting demes. These population profiles are reminiscent of the conceptions of population put forward by Fisher and Wright, respectively. The boundaries of our observation

design set a limit to the generality of this conclusion, which should be confirmed by independent evidence. It is, however, important to consider its potential implications, since these two species are widely used as models in evolutionary biology. The $F_{ST}$ can be interpreted in terms of a decomposition of genetic diversity. Of the total variation that is present within a 30-km area, a local population of *D. simulans* represents only $1 - F_{ST} \approx 97.0\%$ (using the average $F_{ST}$ between sampled sites in this study). The balance is the amount of variation that would be locally and temporarily lost in the overwintering sampling process.

For instance, Veuille *et al.* (2004) showed that, for the 10 loci used in this study, heterozygosity in a *D. simulans* population from Zimbabwe ($H = 0.505$) was larger than that in populations from France ($H = 0.437$). This is in agreement with earlier results from Hamblin and Veuille (1999) showing that non-African populations from this species are less variable than African ones and probably originated through a population bottleneck. If we assume, however, that this species is structured as a metapopulation in France and not in Zimbabwe, then using a single local sample introduces a bias. The value of $H$ is underestimated for Europe and should be corrected as $H/(1 - F_{ST}) \approx 0.450$. This should also occur if the two sexes migrate differently between demes in the metapopulation. For instance, if females migrate less than males, then genetic structuring for mitochondria and for X chromosomes will be increased. This may inflate contrasts when comparing populations from different continents for X-linked genes (Hamblin and Veuille 1999).

A fragmented overwintering population may also introduce changes in the genomic makeup of a species. For instance, homozygotes for deleterious mutations are more likely to appear during strong bottlenecks. This may purge the genome of many deleterious variants, especially lethal genes, and decrease the effect of background selection (Charlesworth *et al.* 1993). A theoretical study by Wright (1937) showed that the steady-state frequency of recessive lethals is smaller in small populations than in large ones (see Wright 1969, pp. 363–366). This model considered strictly recessive lethals. However, many lethal and mildly detrimental alleles in Drosophila have a dominant effect and are thus eliminated as heterozygotes in large populations (Greenberg and Crow 1960). Models taking this into account show that the size of the population still contributes to the load through random fluctuations in allele frequencies (Kimura *et al.* 1963; Nei 1968). However, the mating system could also contribute to the load adjustment by eliminating lethals as homozygotes. Temporal inbreeding in a natural population could thus be a key factor in determining variation patterns.

A model put forward by Kimura and Crow (1970, equation 9.4.12) takes this factor into account. Lethal genes are assumed to follow a gamma distribution $\Gamma(V,S)$, where $V = 4N_e v$ and $S = 4N_e(h + f)$, given that $v$ is the lethal mutation rate, $f$ is the inbreeding coefficient, and $h$ is the dominance disadvantage of lethal heterozygotes. It is assumed that the selection coefficient of lethal homozygotes is $s = 1$. The expected frequency of lethals $E(q) = v/(h + f)$ is independent of the effective population size, but depends on the inbreeding coefficient.

In our results, a striking difference between species lies in $f$, the inbreeding coefficient of populations. Its value is negligible in this study in *D. melanogaster*, but significant in *D. simulans*. A consequence of this would be a larger load of lethal mutations in *D. melanogaster* than in *D. simulans*. We can use the above model to evaluate the magnitude of this effect using realistic values. The average heterozygous disadvantage of a lethal mutation in Drosophila is thought to be $\sim h = 1/40$. Let $f = 0$ in *D. melanogaster* and $f = 1/100$ in *D. simulans*. Then, the average equilibrium frequency of lethals in *D. simulans* would be only 0.715 times its value in *D. melanogaster*. In other words, temporal fluctuations in effective population size would result in an almost 30% decrease of lethals. The breeding structure could also affect the mildly detrimental load. These factors would decrease the effect of background selection. The mutation load is thought to decrease the amount of segregating neutral variation by a factor $f_0 = \exp[-v/(hs + r)]$ (Hudson and Kaplan 1995; Nordborg *et al.* 1996), where $r$ is the local recombination rate. In this expression, the mean selective disadvantage $hs$ of mutant heterozygotes depends only on dominance, whereas our results suggest that it could also be affected by the breeding structure of populations. Provided that the interspecific difference in breeding structure extends over a substantial part of their range, this might be an additional factor involved in differences in genetic variation between the two fruit-fly species. The *D. simulans* genome not only is more variable than that of *D. melanogaster* at the nucleotide level (Aquadro *et al.* 1988; Moriyama and Powell 1996), but also is less variable for natural inversion polymorphisms (Lemeunier and Aulard 1992). In this and in the differential chromosome patterns of molecular variation in its African range (Andolfatto 2001), it resembles *D. melanogaster* X variation more than autosomal variation. A possibility is that the purging effect of temporal variations in population size contributes to all of these effects.

It thus appears that, even though *D. melanogaster* and *D. simulans* are very closely related, a correct interpretation of the differences in their population genetic parameters may require an extensive knowledge of the demographic regime of the populations from which the samples are collected, knowledge which is as yet lacking.

We thank Robert Barbault for encouragement and Richard Lewontin for discussions on the genetics of natural populations. Matthew Cobb and Christian Schlötterer made fruitful comments on the first draft of this article. We thank two anonymous referees for their sub-

stantial and useful criticisms. M.V. expresses special gratitude to Matthew Cobb for 18 years of friendship and comradeship. E.G. was financially supported by a grant of the Conseil Interprofessionnel du Vin de Bordeaux. Her laboratory work was financially supported by Centre National de la Recherche Scientifique (CNRS) Institut d'Ecologie Fondamentale et Appliquée FR 101. M.V. is financially supported by the Groupe de Recherche CNRS 1928 Génomique des Populations and the Plan Pluri-Formation network Populations Fractionnées et Insulaires of the Ecole Pratique des Hautes Etudes.

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Communicating editor: W. Stephan