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Morphological variability and concealed deleterious effects in *Drosophila melanogaster* populations[1]

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Summary. The effects of brother-sister mating on viability of offspring were analyzed in relation to morphological variation of the individuals constituting the sib pairs. *Drosophila melanogaster* populations can be subdivided into 2 distinct groups of individuals that differ in the level of morphological variation and in their response to inbreeding. Genomes free of major deleterious factors appear to be favored by natural selection. A reduction in effective population size due to an environmental shift with consequent selection is thus not expected to be followed by inbreeding depression.

Natural populations of most species of *Drosophila* carry numerous recessive lethals, semilethals, sterility genes, visible mutants, etc., mostly concealed in heterozygous condition[2]. The maintenance in populations of such genes can be attributed to some extent to their heterotic (overdominant) effects. For many years, the fitness of such genes in heterozygotes has been studied by making chromosomes homozygous[2], but the results, which have been widely discussed, are ambiguous, and no clear conclusion can be drawn for natural populations[2]. In the present paper we show that populations of *Drosophila melanogaster* are subdivided into 2 distinct groups that differ in their level of morphological variation. Individuals of sib pairs whose inbred offspring show high viability (no mortality during larval and pupal development) have the smallest morphological variability. Reciprocally, low viability in inbred offspring is associated with parental flies with high morphological variability and high fluctuating asymmetry. This suggests that in the populations studied, genomes free of major deleterious factors are associated with high homeostasis of their carriers and should then be favored by natural purifying selection. This raises the problem of the role and maintenance of heterozygosity and its association with inbreeding depression in natural populations.

The genetic techniques most often used to compare the homozygous and heterozygous effects of deleterious genes or gene complexes involve making chromosomes totally homozygous. Thus, work has been concentrated almost exclusively on the few species of *Drosophila* in which the necessary genetic markers and inversions for this method exist. However, it has been shown recently that certain mutations and lethals reported in natural populations could be the result of interaction between the wild strain studied and the marker strain used to determine the frequency of deleterious genes[3,4]. In addition, a fact that should be underlined is that the general method of producing homozygous chromosomes is an inbred mating system (generally between brothers and sisters), so that, in addition to the chromosome being studied, the entire genome is rendered more homozygous.

In order to study the effects of homozygosity in species that lack the special marker stocks usually used, and in order to avoid the confounding between homozygosity of a particular chromosome and a general increase in homozygosity of the background genotype, we have adopted a different approach. The approach involves studying the relationship between the phenotypic characters of sibs and the viability of their inbred offspring.

Four American populations of *Drosophila melanogaster* (St Paul Q, St Paul P, Sonoma Valley and Pellstom) were established by posting a set of isofemale lines, each of which was derived from a single fertilized female collected in the wild (6 isofemale lines for St Paul Q, 11 for St Paul P, 20 for Sonoma Valley, 25 for Pellstom). These populations were maintained by mass culture at 24 °C for at least 4 generations before the experiments were started.

160–200 F_0 males and females were chosen at random from each posted population and crossed in pairs. The pairs so formed were set up and allowed to lay eggs on a cornmeal molasses medium seeded with live yeast. In order to avoid crowding, 20 eggs from each pair were transferred to vials with fresh medium to allow F_1 progeny to develop. At hatching-time, 1 brother-sister F_1 pair was set up for each progeny group and allowed to lay eggs during 2 successive periods of 10 h each. Two replicates of 50 eggs laid by each sib pair were transferred to new vials to allow F_2 progeny to develop. The F_2 adults produced from the eggs were counted until all had emerged. Egg viability was then estimated for each pair of F_1 sibs by calculating the percentage of fertilized eggs that produced adults. The data from replicates, found to be homogeneous by χ^2 tests, were pooled. In addition, the length of the right wing was measured on each F_1 fly. It should be pointed out that the male and female measured in the F_1 pairs are related (brother and sister) but in no way inbred. Each F_1 sib pair is thus characterized by the wing length of both the male and the female and by the viability of their inbred eggs.

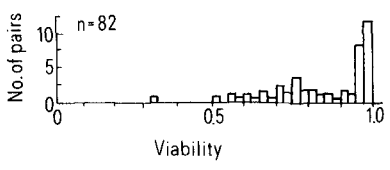

Figure 1. Distribution of the proportion of the fertilized F_2 inbred eggs developing to the adult stage, in progenies from sib pairs, for the Sonoma population. N, number of pairs.

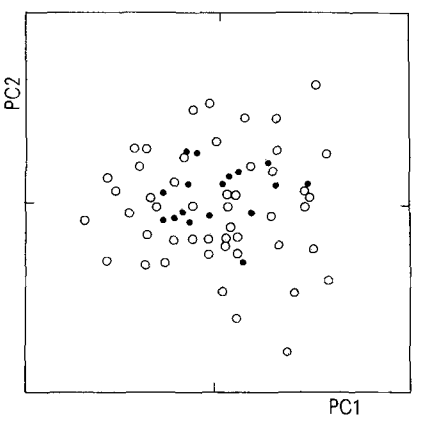

Figure 2. Summary of principal component analysis of sib pairs (each characterized by thorax length and chaeta number of the male and the female) from the Meyzieu population, plotted on the 1st 2 principal components (PC1 and PC2) which together explain about 46% of the total variance. ●, Represents sib pairs with viability offspring above 0.90 (N = 17). ○, Represents sib pairs with viability offspring below 0.90 (N = 48). See text for details. Note the high phenotypic variance of the ○ pairs reflected in the wide scatter of individual morphology.

As shown in figure 1, the distribution of viabilities is bimodal[5,6]. There is one peak at high values (above 0.90) and another peak with a mode ranging from 0.50 to 0.75 depending on the population. There is generally a gap between these 2 modes, so that it is possible to separate sib pairs into 2 classes if we consider the viability value of their inbred offspring: one class for viability greater than or equal to 0.90 and the other for values lower than 0.90. The high offspring-viability sib pairs can thus be considered as free or nearly free of major deleterious factors.

The morphological variability of the F_1 flies for the 2 viability classes appears in the table. Males and females were analyzed separately. Though the differences are not highly significant when tested by a 3-way variance analysis done on the logarithm of the variance[7] ($F = 8.5$; $0.05 < p < 0.10$), the direction of the difference is the same for all of the 8 cases; the flies of the low offspring viability class show higher morphological variation than the flies of the high offspring viability class.

To rule out the possibility of an effect peculiar to wing size, and to determine to what extent the results can be generalized, we have studied a French *Drosophila melanogaster* population (Meyzieu), founded from flies recently caught in the wild. The experimental plan was the same as above. For each F_1 fly, however, we measured the thorax length and the number of sternal chaeta. For these 2 characters considered separately, the flies of the 2 offspring viability classes show the same differences in phenotypic variance as reported above. This result is shown in a different form in figure 2, which represents the results of a principal component analysis applied to characteristics of pairs; each F_1 pair being characterized by the values of the 2 morphological characters in both the male and the female. The pairs with high viability of their offspring (black dot) are clearly less morphologically variable as judged by the 1st 2 principal components. Thus, a population can be subdivided into 2 groups, based on the viability of offspring of sib pairs, and these groups differ in their level of morphological variation. This dissection of a population is repeatable in the 5 geographic localities studied independently in time (4 in America and 1 in France) and for the different characters studied. Moreover, neither of the 2 groups is negligible in its proportion in the population. As shown in the table, the high offspring viability group ranges from 24 to 56% of the population. This phenomenon is thus of great importance for population.

What is the meaning of the discrepancy between the 2 groups? As far as wing size is concerned, deleterious genes have been shown to increase wing size in *Drosophila* when heterozygous[8]. However, there was no significant effect of mean wing size in the variance analysis of the American populations ($F < 1$). In addition, such an explanation can hardly explain the effects that were observed on the variance of the size of the thorax and the number of chaeta in the French population for which no effect on the mean was detected. An explanation of the results involving inversions does not apply since it has been reported that there is no significant difference in the proportion of deleterious genes between inverted and inversion-free chromosomes[9]. Our results are in agreement, however, with experiments dealing with homozygous chromosomes and showing that single lethal heterozygotes are inferior to lethal-free heterozygotes with respect to fitness as a whole[9]. Unfortunately, no data on morphology is available on such lethal-free individuals.

It is generally admitted that smaller morphological variance and high fitness, which are criteria of high homeostasis, characterize highly heterozygous individuals[10-16]. To test this hypothesis further, we measured the fluctuating asymmetry (FA) of wing length in the Sonoma population. This measure of developmental homeostasis is expressed in terms of variance of the difference in score between left and right sides[15-18]. The values of FA in the high and low viability offspring classes are 0.0009 and 0.0017 in males ($0.01 < p < 0.05$) and 0.0009 and 0.0010 in females (nonsignificant). Hence, our high offspring viability F_1 individuals, with small morphological variability and a low FA (at least in the population studied) can be regarded as being highly heterozygous. One might postulate that the higher the degree of heterozygosity of an individual, the higher the probability that deleterious factors might be concealed in its genome. This implies that a low morphological variability of the F_1 individuals should be associated with high inbreeding depression in the offspring. The opposite association is observed in our experiment. This, in turn, favors the 'classical' view in population genetics, which predicts that chromosomes free of major deleterious factors constitute the 'best' genotype[2]. The individuals that carry major deleterious factors in heterozygous state are selected against by purifying selection. This reinforces the idea of Sved and Ayala[19] that the existence of a large number of chromosomes with little or no reduction in viability in homozygous condition is incompatible with the hypothesis of heterozygote superiority at a large number of loci. That view is in disagreement with recent observations of a higher developmental homeostasis in individuals highly heterozygous for enzyme loci[10-12]. Whether or not there is a correspondence between heterozygosity for the enzyme loci and for deleterious factors clearly warrants further investigation.

If selection tends to favor overall optimal genotypes that meet all vicissitudes of the environment, the highly homeostatic individuals must be selected after an environmental shift. We have shown above that this group shows no inbreeding depression in its offspring. Thus, though a reduction in effective population size may follow the selection process, no inbreeding depression would be expected in the immediately following generations. This could help explain recent observations in the Great Tit in which 'fitter' individuals have a higher chance of becoming involved in inbreeding[20]. From the results of our study, no drastic deleterious effect should be observed in their offspring.

Phenotypic variance of wing size for males and females grouped into 2 classes of viability value of their inbred offspring resulting from sib crosses

Populations	Viability			
	N	Less than 0.90	N	Greater than or equal to 0.90
Males				
Sonoma valley	39	0.014	50	0.008
Pellstrom	46	0.014	30	0.013
St Paul P	47	0.017	15	0.008
St Paul Q	47	0.006	23	0.005
Females				
Sonoma valley	39	0.020	50	0.016
Pellstrom	45	0.020	29	0.011
St Paul P	47	0.015	15	0.008
St Paul Q	49	0.013	22	0.012

N, sample size. For the meaning of the viability classes, see text.

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Lens forming transformations in larval *Xenopus laevis* induced by denatured eye-cup or its whole protein complement[1]

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Summary. Implants of lensectomized eye placed between the outer and inner cornea, denatured by ethanol treatment and implants of total protein pellets from homogenates of lensectomized eyes, induce lens-forming transformations of the outer cornea of larval *Xenopus laevis.*

In larval *Xenopus laevis* lens regeneration from the outer cornea is known to require the presence of the eye-cup[3,4]. It has been established by subsequent research that a necessary and sufficient condition for the outer cornea to produce a lens is for it to be in direct communication with the vitreous chamber environment. In order to achieve this, the 2 mechanical barriers consisting of the lens and the inner cornea, which in a normal eye lie between the outer cornea and the vitreous chamber, must be by-passed[5-7]. Damage to the outer cornea alone is not enough to trigger the regenerative process[6,8].

This suggests that a lens-inducing factor is probably present in the vitreous chamber and that lens regeneration is due to this factor spreading from the vitreous chamber to the outer cornea. Recent research indicates that the factor is produced in the neural retina[9-11]. Although we speak about a factor it cannot be excluded that there is more than 1 lens-inducing factor. The present work aims at providing more

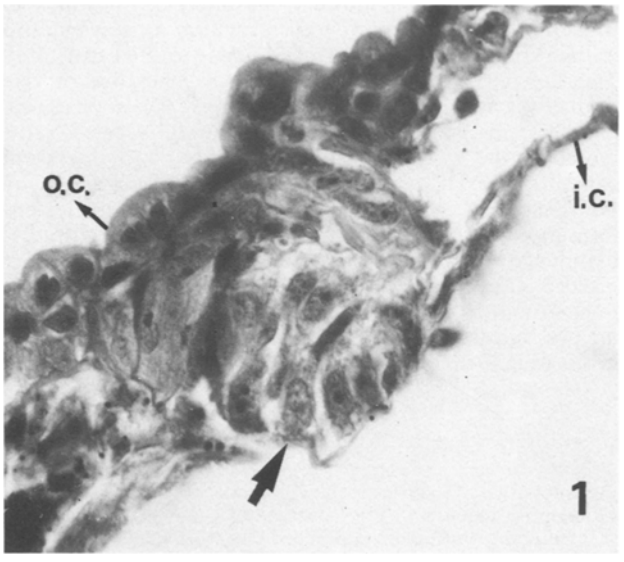

Figure 1. Lens vesicle at early stage 4 (arrow) developing between the inner (i.c.) and outer cornea (o.c.) 5 days after implantation of lensectomized eye devitalized 24 h after lensectomy (experiment 1). × 700.

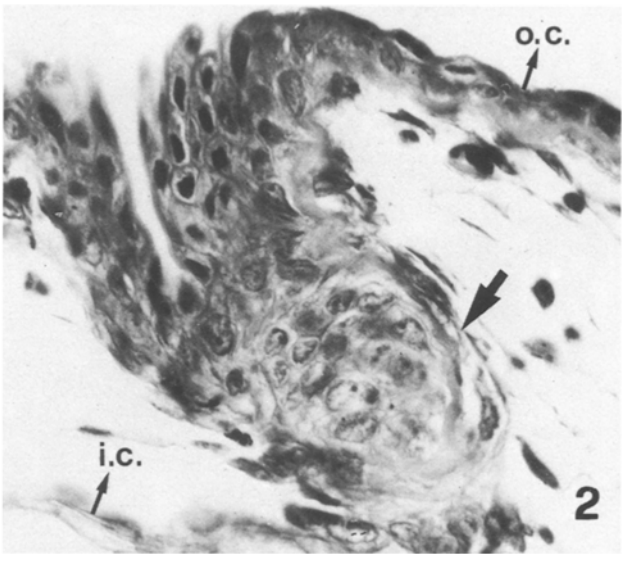

Figure 2. Lens vesicle at late stage 3 (arrow) developing between the inner (i.c.) and outer cornea (o.c.) 9 days after implantation of lensectomized eye devitalized immediately after lensectomy (experiment 2). × 550.