INTROGRESSION IN LABORATORY POPULATIONS OF *DROSOPHILA PERSIMILIS* AND *D. PSEUDOOBSCURA*

LEIGH VAN VALEN

Zoology Department, University College, London

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1. INTRODUCTION

Drosophila persimilis and *D. pseudoobscura* are sibling species almost completely isolated reproductively from each other (see the discussion by Dobzhansky, 1951). Only two hybrid individuals out of more than 50,000 individuals chromosomally examined have been found in nature (Dobzhansky, personal communication). Sexual isolation is present but incomplete (review by Dobzhansky, 1951).

The present experiment was undertaken in order to see what would happen when *D. persimilis* and *D. pseudoobscura* were put together in population cages, with a greater-than-normal advantage for hybrids. Three results were possible: elimination of one of the species, co-existence of both species, and the formation of a hybrid swarm by introgression. If the species had coexisted for a sufficient period it is possible that selection for reproductive isolation would have occurred, as it did in the differently designed experiment of Koopman (1950) with the same mutants of the same species. However, only the first and third alternatives were actually found.

2. PROCEDURE

The recessive mutants *orange* (*or*) of *D. persimilis* and *glass* (*gl*) of *D. pseudoobscura* were used. Both these mutants affect the eyes. *or* is located near the terminal heterochromatin containing the centromere of the third chromosome and *gl* on the distal part of the second chromosome (Tan, 1937). The hybrid between them has eyes with a visibly normal phenotype and, as will be shown below, is more fit in at least one respect than either homozygote. Nevertheless, as shown by Lancefield (1929) and others, its fertility is greatly impaired. Each of these mutant strains was outcrossed to two wild-type laboratory strains with the same gene sequence on the third chromosome, and the mutants were re-extracted.

Two hundred mutant males and 200 non-virgin mutant females of each species were placed on 31st July 1961, into each of six plastic population cages of the kind described by Strickberger (1963). Three of these cages (V-15, V-16 and V-17) were kept at $16^{\circ} \pm 1^{\circ}$ C., and three (V-18, V-19 and V-20) were kept at $25^{\circ} \pm 1^{\circ}$ C. This was done because sexual isolation between these species has been reported (Mayr and Dobzhansky, 1945) to be lower at $16\frac{1}{2}^{\circ}$ than at $24\frac{1}{2}^{\circ}$, and *D. persimilis* thrives better at 16° than at 25° (Dobzhansky, 1935). *D. pseudoobscura* also thrives somewhat better at the lower temperature, but its difference is not as great.

Since the development time of *D. persimilis* in bottles averages a few hours longer than that of *D. pseudoobscura* (Poulson, 1934), the period each food cup was left in the cage before replacement was varied. In V-18 it was 35 days; in V-19, 40 days; in V-20, 45 days; in V-15, 52 days; in V-16, 60 days; and in V-17,

67 days. The generation length of *D. pseudoobscura* in comparable population cages has been estimated at about 29 days for 25° and about 43 days for 15½° (Van Valen, L. Levine and Beardmore, 1963). There is no adequate published estimate of the generation length of *D. persimilis* in population cages, and there is no clear indication in the present experiment of an influence of the different lengths of food cup cycles.

Periodic egg samples and occasional counts of the entire adult populations were taken. The eggs were raised under optimal conditions at 19° except where otherwise stated, and all the adults that emerged were scored for eye colour. On two occasions, most of the larvæ from supplementary bottles were dissected and their salivary chromosomes examined. Since the flies with different eye colours develop at different mean rates (see below), larvæ maturing at different times were dissected in numbers roughly proportional to those maturing in each interval.

3. MUTANT FREQUENCIES

As can be seen from tables 1 and 2, the populations at 16° behaved almost identically, with the early elimination of all *D. persimilis* and

TABLE 1

Results of egg samples from the populations. In each case the left number represents phenotypically orange flies, the middle number wildtype, and the right number glass flies

First day of sample	V-15	V-16	V-17	V-18	V-19	V-20
9*	14-0-392	26-23-181	55-10-105	83-5-293	74-6-175	120-7-186
9*	...	...	...	73-9-135†	124-1-133†	...
25	...	...	...	0-185-591	...	15-99-389
46	2-3-192	1-0-134	0-0-147	...	...	...
53	...	...	...	...	0-3-9	0-22-56
73	...	...	...	0-7-32	0-241-117	0-43-50
86	0-0-47	0-0-59	1-0-69	...	...	...
97	...	...	...	0-18-48	...	0-11-36
114	...	...	...	0-0-0	0-79-33	0-30-58
124	0-0-411	0-0-389	0-0-474	...	...	...
134	...	...	...	0-2-21	1-426-133	0-27-31
154	...	...	...	1-4-8	0-117-22	0-32-27
175	...	...	...	0-1-10	0-188-54	0-66-53
196	...	...	...	...	0-156-22	...
214	...	...	...	0-0-53	...	...
237	...	...	...	...	0-376-15	0-11-27
256	...	...	...	...	0-472-48	...
275	...	...	...	...	0-1134-93	...
288	...	...	...	...	0-342-18	...

* Eggs laid by the parental flies, which were non-virgin on entering the cages.

† Eggs taken from side instead of top of food.

visible hybrids. Examination of a total of 348 haploid sets of salivary chromosomes from the three cages, from eggs laid on the 124th day, showed a single second chromosome of *D. persimilis*, all the other X and second chromosomes being completely *D. pseudoobscura*.

A greater or lesser degree of introgression occurred in all three populations at 25°. Although it can be seen from tables 1 and 2 that pure *D. persimilis* was eliminated rather rapidly, wild-type flies survived in each cage to its termination and made up nine-tenths or

more of population V-19 in its last two or three months. Males and females were recorded separately but never gave discordant frequencies.

The occurrence of introgression at 25° while *D. persimilis* was eliminated at 16° was unexpected and remains unexplained.

The egg samples do not provide an entirely accurate picture of the phenotype frequencies in the population cages. The adult samples are usually not much different, but near the end of the V-18 population wild-type flies were much more frequent in the cage than in the egg samples. Furthermore, egg samples from the parental generation of V-18 and V-19 differed somewhat in composition according to whether they were taken from the top of the food cup, as in all subsequent samples, or from the side where part of the food has been removed,

TABLE 2

Counts of adults in the population cages. Order of phenotypes as in table 1

Day of count	V-15	V-16	V-17	V-18	V-19	V-20
24	...	...	...	...	43-30-148	...
39	...	...	...	7-93-186	...	...
76	...	...	...	...	2-389-124	...
145	0-0-893	0-0-937	0-0-725	...	...	...
180	...	...	...	0-11-126	...	...
195	...	...	...	...	0-470-74	0-27-22
218	...	...	...	0-12-96	...	...
261	...	...	...	...	...	0-24-63
310	...	...	...	...	0-721-73	...

where most of the eggs that remain in the cage are laid. *D. persimilis* seemed to prefer the latter region.

The samples from the parental generation (where only the mutant frequencies are valid because of non-virginity of the introduced parents) indicate that there was some selection among the adults against *D. persimilis* in all or nearly all cages. The form of this selection is unknown.

4. CHROMOSOME FREQUENCIES

Since only the X and second chromosomes of *D. pseudoobscura* and *D. persimilis* can be distinguished cytologically (Tan, 1935; Koller, 1936; Sturtevant and Dobzhansky, 1936; Dobzhansky and Sturtevant, 1938; Dobzhansky, 1944), cytological information was available on the changes in frequency of only these chromosomes. In addition, however, the gene *or* gives some information about the third chromosome. Since some crossing over occurs in *D. pseudoobscura* despite the presence of even moderately long or complex inversions on the same chromosome (Dobzhansky and Epling, 1948; R. P. Levine, 1956; Sturtevant and Dobzhansky, 1936; Dobzhansky and Sturtevant, 1938; also see below), X and second chromosomes,

cytologically of one species, may in fact contain some genes of the other. The strains of the species used differ cytologically by an inversion on each arm of the X chromosome and one on the rod-shaped second chromosome.

On the 134th day an egg sample was taken from each 25° cage for chromosome analysis. The results are shown in table 3. The numbers of X chromosomes are smaller than those of second chromosomes because of the presence of males, which have only a single X chromosome. Frequencies of each chromosomal association (e.g. *pseudoobscura* XL, *persimilis* XR, hybrid II) are not given because in no case was the association between X and II other than random,

TABLE 3
Results of chromosome samples of the populations

	XL		XR		II	
	<i>pers.</i>	<i>pseud.</i>	<i>pers.</i>	<i>pseud.</i>	<i>pers.</i>	<i>pseud.</i>
Day 134						
V-18 . . .	2	17	1	18	3	25
V-19 . . .	31	47	34	44	69	31
V-20 . . .	13	39	11	41	39	37
Day 275						
V-19 . . .	35	38	31	42	73	27

although in every case a positive association between XL and XR was possible (P always less than 0.2, usually much less).

The frequencies for the two arms of the X chromosome are comparable in each population, and show a moderate to marked reduction in the *D. persimilis* gene arrangements. In populations V-19 and V-20, however, there is a significantly greater proportion of *D. persimilis* second chromosomes than of *D. persimilis* X chromosomes.

The two estimates of the frequency of *D. pseudoobscura* second chromosomes provided by cytological analysis and examination of eye colour are in general agreement but are nevertheless significantly different for both V-19 and V-20. The latter shows that crossing over has occurred between *gl* and the inversion and that the chromosome segments containing these elements have different adaptive values in the mixed population genome. In each case the segment with the inversion is less abundant than the segment with *gl*. This is true whether the Hardy-Weinberg formula or the observed chromosome pairings (see below) are used to estimate the frequency of heterozygotes for *gl*.

In each chromosome sample (except those of V-18, where the test is insensitive) the heterokaryotypes were significantly less frequent than expected by the Hardy-Weinberg formula, indicating the probable

presence of an isolating mechanism, presumably in this case sexual isolation between the parents, acting before the late third larval instar. This is the reverse of the result obtained by Mettler (1957). It might seem that the intensity of this presumed sexual isolation could be crudely estimated for V-19 and V-20 by dividing the number of heterokaryotypes by the number of homokaryotypes (*cf.* Merrell, 1950). However, this estimate is biased because (1) the parental homokaryotypes were of unequal frequency and (2) some of the parents were presumably heterokaryotypes, but the relative fertility and mating propensities of these are unknown. The first bias can be overcome statistically but the second cannot without additional information that is not available for this generation.

Eight virgin *gl* flies from V-19 were put as pairs into four vials. Their offspring were raised in the usual way and nine larvæ from each pair were dissected. In at least two and at most four of the parental flies a *persimilis* second chromosome gene arrangement was present in heterozygous condition, thus proving the occurrence of this gene arrangement and the *pseudoobscura* mutant *gl* on the same chromosome. Much the most probable cause of this is crossing over.

A second sample of chromosomes was taken on the 275th day from V-19, the only population remaining. As in the earlier sample, the association between X and II was random but between XL and XR it was strongly positive. The frequencies of *persimilis* XL and XR were not markedly different from their earlier values, suggesting the establishment of an equilibrium between 40 and 50 per cent. The cause of this apparent equilibrium is unknown, but Mettler (1957) found a similar results in his cage 6 (see also Wallace (1948) and Bennett (1958)). Despite the drop in the frequency of *gl*, the frequency of the *pseudoobscura* second chromosome gene arrangement was not significantly changed, suggesting an equilibrium at about 30 per cent. This is about the frequency of *gl* if the Hardy-Weinberg formula were applicable. It does in fact appear to be applicable at this time, since the frequencies of heterokaryotypes are now not significantly different from those expected (χ^2 computed both independently and combined for the three classes).

5. SEXUAL ISOLATION

Virgin flies raised at 25° from eggs and aged for about a week were used for tests of sexual isolation against the mutant strains of each pure species (raised for two generations at 25°). Ten males and five females of each of two kinds were placed in a creamer for three days at 25°. The females were then dissected and the presence or absence of sperm in the spermathecae and ventral receptacles noted. Merrell (1954) has validated this method for these two species at 21°.

Adults from the egg sample of V-19 on the 275th day were used for estimates of sexual isolation, together with flies from the parental strains hatching at the same time. The crosses and the results are

summarised in table 4. In each creamer five homogamic females were present together with the five heterogamic females indicated. The small number of *glass* females from the population cage prevented further crosses.

The results are striking. There is no indication whatever of sexual isolation between the hybrid population and either parental species, despite the strong isolation between the parental species. This is reminiscent of the results of Mayr (1946), who found a generally

TABLE 4

Results of sexual isolation tests between flies from V-19 and stock flies. The first fraction of each pair represents homogamic matings, the second heterogamic matings in the same creamers. Each fraction is the number found inseminated divided by the total number dissected

	Heterogamic females		
	<i>D. pers.</i> , or	cage, +	<i>D. pseud.</i> , gl
Males :			
<i>D. pers.</i> , or . . .	...	$\frac{18}{33}, \frac{15}{31}$	$\frac{17}{31}, \frac{2}{30}$
cage, + . . .	$\frac{23}{30}, \frac{25}{31}$	...	$\frac{20}{33}, \frac{23}{30}$
cage, gl . . .	$\frac{16}{21}, \frac{15}{23}$	...	$\frac{15}{22}, \frac{13}{21}$
<i>D. pseud.</i> , gl . . .	$\frac{24}{33}, \frac{4}{31}$	$\frac{21}{30}, \frac{24}{32}$	...

similar situation for F_1 hybrids between the same species, and of Birch (1961), who found a similar but less extreme breakdown of isolation in a laboratory hybrid swarm of *Dacus*. A formal test of significance seems unnecessary here.

6. MORPHOLOGY

Reed, Williams and Chadwick (1942) discovered that a theoretically derived morphological index adequately separated all specimens tested of *D. persimilis* and *D. pseudoobscura*, when these specimens were raised under comparable conditions. When the index was used in combination with sex comb number (Mather and Dobzhansky, 1939) an even better separation was found. The index is the product of the wing area by the cube of the wing length.

Wild-type males from V-19, derived by the egg sample of the 275th day, were compared with the parental mutant strains for the wing index. All were raised at 25° in optimal conditions. Wing area was taken to be the product of wing length by wing breadth, since shape aside from these components is nearly uniform. The

results are shown in table 5, the wing index being given in arbitrary units.

For each character the flies from V-19 are intermediate between those of the pure species. In the wing index they are slightly closer to *D. persimilis* and in the sex comb count to *D. pseudoobscura*. The *gl* flies from the cage are ostensibly but not significantly closer than the wild-type flies to *D. pseudoobscura* in both characters. The flies from the cage are markedly and significantly more variable than the pure species in both characters, suggesting that an integrated population genotype has not yet become completely established. An alternative possibility to explain this greater variance, that the population cage

TABLE 5

Comparison of flies from V-19 with stock flies, by a wing index and number of bristles in both sex combs. s represents the standard deviation of the sample

	Index		Sex combs		No. measured
	$\bar{X}$	<i>s</i>	$\bar{X}$	<i>s</i>	
<i>D. pers.</i> , <i>or</i>	7.43±0.06	0.40	9.83±0.12	0.77	40
cage, + ...	6.85±0.10	0.61	10.74±0.16	0.97	40
cage, <i>gl</i>	6.92±0.11	0.53	10.81±0.22	1.12	25
<i>D. pseud.</i> , <i>gl</i>	6.12±0.05	0.31	11.32±0.13	0.83	40

has diverse environments that flies of different wing and sex comb phenotypes exploit, has been rejected as a result of counting the teeth of sex combs on 40 *D. pseudoobscura* taken as eggs from a population cage (173-22) and raised under comparable conditions. The population standard deviation was 0.78, and the mean was comparable.

7. DEVELOPMENT TIMES

Examination of the day-by-day counts of the flies from egg samples made it clear that those homozygous for *or* and *gl* were slower in development than the wild-type ones. Table 6 summarises the data, giving the mean intra-bottle differences between the development times of the different phenotypes, using of course only those bottles in which both the classes being compared emerged. All differences are significant at the 5 per cent. level or lower. No development times were estimated for flies emerging from food cups in the cages, but the breakdown for crowded and uncrowded cultures accentuates the differences in development time.

In a population cage the selective advantage of a short development time is much greater than would be predicted by merely estimating the time an additional generation would appear for the faster genotype. This is because the first flies emerging from their

puparia are the first to lay eggs, and their larvæ will be larger than those from eggs laid later. Later eggs may not be laid at all because of the churning mass of food and larvæ usually occupying the cup.

TABLE 6

Within-bottle comparisons of development times for three different phenotypes taken as eggs from the population cages. Each number represents the mean additional time (in days) that the phenotype on the left in each pair took in comparison to the phenotype on the right. Crowded cultures here mean those with over 200 adults of all phenotypes emerging

	Crowded		Uncrowded	
	Males	Females	Males	Females
<i>or</i> , <i>gl</i>	1.40	1.10	0.60	0.72
<i>or</i> , + ...	1.59	2.61	0.37	1.68
<i>gl</i> , + ...	0.63	1.03	0.52	0.66

In the fiercely competitive situation of a population cage food cup it is presumed that larger larvæ have a decided advantage.

8. DISCUSSION

The number of individuals recorded in each sample in table 1 is very roughly proportional to the number of adults in the population, as judged by adult counts (table 2) and visual examination of the cages, even though the number of bottles counted is not always the same. After about the second month the adult population size of V-19 became and remained noticeably larger than that of V-18 and V-20.

The small size of populations V-18 and V-20 may reflect the action of both the advantage of development time (and perhaps others) of wild-type flies and some of the isolating mechanisms reviewed by Dobzhansky (1951). The backcross progeny are weakened and often sterile; this is reflected in the high proportion (usually more than half, in one case all) of the eggs in samples from V20 and especially V-18 that failed to develop. If most of the flies that survive are hybrids, perhaps because of the situation described in the preceding section, their progeny will be fewer than if they are of the pure species. V-19 also had a small adult population size for a while, but soon recovered. This recovery may have been due to a reorganisation of the genome that permitted chromosomes of both species to remain without causing undue harm to their carriers.

The differences between the three populations in the frequencies of visible and cytological chromosome markers are perhaps a result of the random occurrence of gene combinations that provided different

receptiveness to heterospecific chromosomes in each population. It is known (Dobzhansky and Boche, 1933) that there is genetic variability within both *D. pseudoobscura* and *D. persimilis* with respect to resistance to testis degeneration in F_1 hybrids between the two species. It is plausible but unproved that similar variability exists with respect to resistance to other aspects of hybrid breakdown and sterility. The divergent courses followed by the three populations at 25° may well be due to the occurrence of different gene combinations in the three populations, with a resultant divergence in selective trends and available adaptive peaks (cf. Dobzhansky and Pavlovsky, 1957; Dobzhansky and Spassky, 1962; and Van Valen, L. Levine and Beardmore, 1963).

9. SUMMARY

Mutant *D. pseudoobscura* and *D. persimilis* were put into population cages at 16° C. and 25° and left undisturbed. *D. persimilis* was soon eliminated at 16°, but at 25° a hybrid swarm was formed in each of the three populations. These hybrid swarms were analysed by use of the mutant markers, the chromosomal differences between the species, sexual isolation, and morphology. The populations were in every case intermediate between the parental species. An apparent equilibrium in the X as well as the second chromosome was present. The experimental flies were sexually isolated from neither parental species, and were morphologically more variable. The three populations at 25° were not identical, perhaps because of different courses taken in the reorganisation of their genomes.

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