INCREASE IN GENETIC VARIANCE FOLLOWING A POPULATION BOTTLENECK

Severe population bottlenecks may produce genetic effects of importance in the process of species formation (Mayr 1954; Lewis and Roberts 1956; Carson 1971). Although this idea has been challenged (Barton and Charlesworth 1984), it is supported by considerable indirect evidence (e.g., Gottlieb 1973; Carson and Templeton 1984). Some experiments (Powell 1978; Ringo et al. 1985) and theories (Nei et al. 1975; Templeton 1980) imply that genetic shifts may accompany a founder event. Here we report data on an experimental laboratory population indicating that reduction in population size may sometimes be accompanied by an *increase* rather than a *decrease* in genetic variance accompanying stochastic events associated with the bottleneck. If followed by renewed and realigned natural selection, a substantial shift in genome organization might ensue.

A vigorous population of the endemic Hawaiian species *Drosophila silvestris* (U28T2) has been maintained in the laboratory since 1977. This population was established from a single, naturally inseminated wild female. Chromosomal-inversion polymorphism is widespread in the natural populations of this species. Three natural, spatially independent inversions in chromosome 4 were found segregating in this population in 1980; they have persisted thereafter at high frequencies. The inversion loops are illustrated in figure 1: $\mathbf{k}^2$ (right) is distal, **t** is central, and $\mathbf{l}^2$ is proximal.

When this population was used for a behavioral study in 1984–1985 (Carson 1987*a*), a considerable number of females and males were test-crossed in order to infer which of the eight possible haplotype combinations of the three inversions were segregating in the population. These tests showed that only three haplotypes were being carried by adult flies at this time: (1) $\mathbf{k}^2$ **t** +, (2) + + $\mathbf{l}^2$, and (3) + + + (fig. 2). Frequencies at this time are shown in table 1 (1985, first row). Crossing-over between the inversions in doubly and triply heterozygous flies both from U28T2 and from other strains is absent in males (n = 1613 gametes observed) and is rare in females: 0.004 between $\mathbf{k}^2$ and **t** (n = 1153) and 0.003 between **t** and $\mathbf{l}^2$ (n = 1015). Except for a single instance, the rare recombinant haplotypes were observed only in larvae. Because of the spatial distribution of the inversions, the haplotypes include large sections of the chromosome that undergo crossing-over only rarely.

Another sample of test-crossed adults, taken in September 1986, showed that these haplotype frequencies had not changed, either quantitatively or qualitatively (table 1, second row). In December 1986, however, the population was subjected to sustained high temperatures caused by an equipment malfunction in the insect-

Am. Nat. 1989. Vol. 134, pp. 668–673.

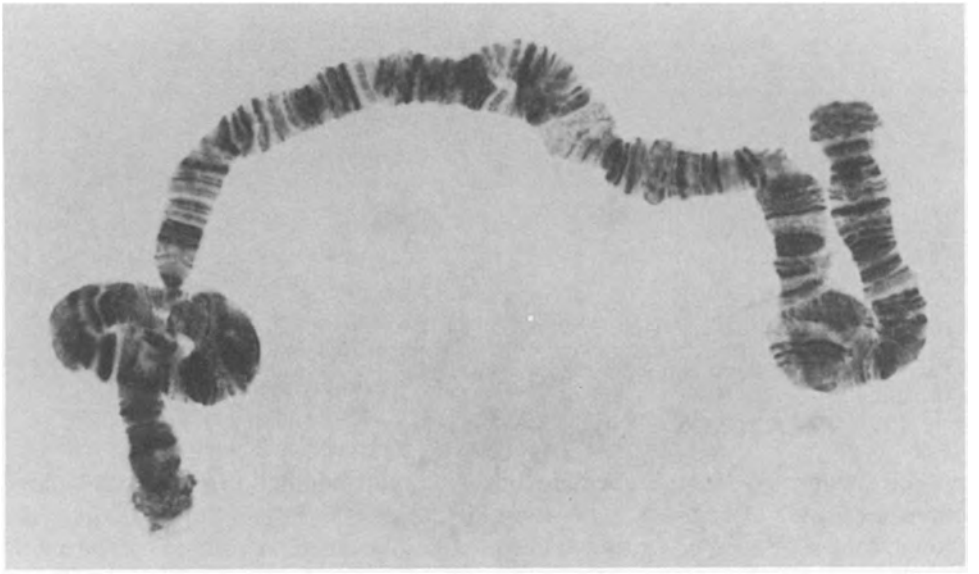

FIG. 1.—Chromosome 4 of *Drosophila silvestris* showing three heterozygous inversions. *Right*, Distal end with inversion k^2; inversion t is central; *left*, proximal end with inversion l^2.

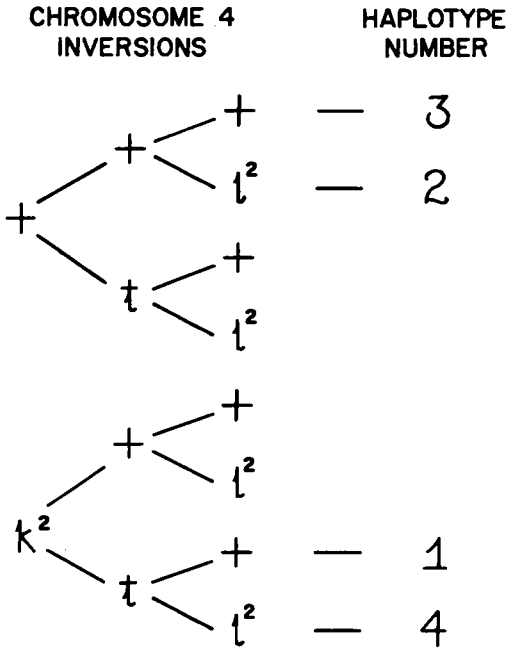

FIG. 2.—Diagram of the eight possible haplotypes formed by the three independent inversions in chromosome 4. Numbers 1, 2, and 3 have existed throughout the history of the population; number 4 is a new haplotype that arose at or about the time of the bottleneck. The others have not been observed in breeding adults, except for a single occurrence of $k^2 + l^2$ in one female.

TABLE 1

FREQUENCIES OF CHROMOSOME-4 HAPLOTYPES OBSERVED IN ADULTS BEFORE (1985 AND 1986) AND AFTER (1987 AND 1988) A POPULATION BOTTLENECK

		HAPLOTYPE			
		1	2	3	4
SAMPLE	N	k^2t +	+ + l^2	+ + +	$k^2t\ l^2$
1985	274	0.547	0.336	0.117	0
1986	114	0.640	0.290	0.070	0
1987	172	0.093	0.320	0.256	0.331
1988	112	0.143	0.339	0.232	0.286

NOTE.—1985 does not differ from 1986: $\chi^2 = 3.44$, df = 2, $P = 0.18$. 1987 does not differ from 1988: $\chi^2 = 2.18$, df = 3, $P = 0.54$.

rearing facility. When the accident occurred, the population consisted almost entirely of adult specimens. The approximately 200 adults were not killed, although they produced no progeny. The population was reconstituted by rearing to adulthood a small number of larvae that proved to have escaped the sterilizing effects of the heat; there were possibly as few as three of each sex.

The specimens that were larvae at the time of the December heat episode emerged as adults in approximately the third week of January 1987. Four weeks later, in February, the first adult progeny produced by this small number of flies eclosed. This is a normal life-cycle interval for this species. Forty to 50 adults were obtained and were set up in a breeding jar on April 1, 1987. From then on, a normal population size of about 200 adults was restored and has been maintained subsequently. A new recombinant haplotype ($k^2\ t\ l^2$; fig. 2, #4; table 1) was first recognized in a small larval sample taken in May 1987; these were larvae of the second generation after the lab accident. When the population was fully tested 6 mo later in November 1987 (about 1 yr after the bottleneck), this new haplotype exhibited a frequency of about 30%; this was unchanged a year later (table 1, last two rows).

Before the bottleneck, as reported earlier (Carson 1987*a*), the three haplotypes were observed to form 6 types of zygotes, the expected 3 heterokaryotypes and 3 homokaryotypes. In breeding adults, triply heterokaryotypic individuals (zygotic combination 1/2 appeared to have high fitness, as measured by survival and sexual activity. Following the bottleneck and the appearance of the new haplotype, all 10 possible zygotic combinations formed by these haplotypes (6 heterokaryotypes and 4 homokaryotypes) were observed in adults except + + + / + + +. Their frequencies, first observed in 1987, were unchanged a year later (table 2). Thus, there was manifested, and virtually realized, a 50% increase in zygotic combinations in adult flies; this represents a substantial increase in genetic variance.

Table 2 gives the distribution of the new set of observed karyotypes compared with the expected distribution as estimated from haplotype frequencies. Zygotic combinations 4/3 and 4/2, both involving the new haplotype, are especially fre-

TABLE 2

ZYGOTIC COMBINATIONS OF HAPLOTYPES (1, 2, 3, 4) AS OBSERVED IN BREEDING ADULTS TAKEN FROM THE EXPERIMENTAL POPULATION IN 1987 AND 1988, FOLLOWING THE BOTTLENECK

Zygotic Combination	Number Observed	Number Expected*
	HETEROKARYOTYPES	
4/3	38	21.9
4/2	40	29.1
2/3	23	22.9
1/2	12	10.5
1/3	9	7.9
1/4	9	10.0
	HOMOKARYOTYPES	
4/4	1	13.9
1/1	1	1.8
2/2	9	15.2
3/3	0	8.6
TOTAL	142	141.8

NOTE.—Homogeneity tests of distribution: 1987 males and females, $G_H = 1.68$, df = 9, $P \gg 0.25$. 1988 males and females, $G_H = 5.90$, df = 9, $P \gg 0.25$. 1987 and 1988 samples, $G_H = 3.69$, df = 9, $P \gg 0.25$.

* Goodness of fit: $G = 48.15$, df = 8, $P < 0.005$. Expectations were estimated from haplotype frequencies.

quent in breeding adults, whereas 1/2, which was in excess before the bottleneck, is no longer above expectation. Although the triple heterozygotes 4/3 and 1/2 (the latter is depicted in fig. 1) have an identical appearance in the synapsed chromosome as observed under the microscope, the data indicate important differences in fitness between these karyotypes following the bottleneck.

The increase in haplotype number from three to four appears to be due to a rare crossover between inversions t and l^2, arising out of a triple heterokaryotype and yielding the new haplotype. This novel chromosome could then have achieved a high frequency through random drift imposed by the bottleneck. Nevertheless, it has been maintained in the population for 2 yr, that is, about 10 generations in *D. silvestris*, for which the time of a single generation at 18°C is about 11 wk. The new haplotype has existed over this time at about 30% in the population at equilibrium frequencies favoring heterokaryotypes 4/3 and 4/2 (table 2). This strongly implies a role for natural selection in its maintenance.

The observed change appears to be a reorganizational one, caused by crossing-over. The new components have been integrated by selection into an altered zygotic fitness system. Such a change in genetic variance, involving a restructuring of the balance relationships of one of the five major chromosomes, would appear to be of much greater significance than simple variance changes resulting

from a mutational process, for example, one that involves change in only a single allele.

In summary, a stochastic change in chromosomal organization by recombination, appearing at or about the time of the bottleneck, has permitted the release of novel genetic variance to the subsequent action of natural selection. These events appear to be similar to the increase in genetic variance following a very small bottleneck observed in the recent experiments of Bryant et al. (1986) and Bryant and Meffert (1988). The theoretical models of Goodnight (1987, 1988) also predict variance increase under similar conditions.

In all the above cases, there has been an unexpected *increase* in genetic variance following the bottleneck. Since the conventional view stresses the opposite effect, that is, a *loss* in genetic variance (i.e., one or more alleles) by random drift at a bottleneck, the possibility of variance gain is of special interest in population genetics and evolution. This type of stochastic change in organization, followed by renewed selection, is a prominent feature of some theories of species formation (e.g., Carson 1987*b*). The relationship of these population-genetic shifts to events that might occur during the process of species formation has not been established. Nevertheless, our findings suggest that the subject can at least be brought under experimental scrutiny at the population level.

ACKNOWLEDGMENTS

We thank L. T. Teramoto, who cared for the population and made many of the chromosome smears. K. Y. Kaneshiro, Director of the Hawaiian Evolutionary Biology Program, University of Hawaii, provided logistic support. We thank T. W. Lyttle for advice on statistical procedures and for reading the manuscript. The work was supported by a grant from the National Science Foundation (BSR 84-15633).

LITERATURE CITED

Barton, N. H., and B. Charlesworth. 1984. Genetic revolutions, founder effects, and speciation. Annu. Rev. Ecol. Syst. 15:133–164.

Bryant, E. H., and L. M. Meffert. 1988. Effect of an experimental bottleneck on morphological integration in the housefly. Evolution 42:698–707.

Bryant, E. H., S. A. McCommas, and L. M. Combs. 1986. The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. Genetics 114:1191–1211.

Carson, H. L. 1971. Speciation and the founder principle. Stadler Genet. Symp. 3:51–70.

———. 1987*a*. High fitness of heterokaryotypic individuals segregating naturally within a long-standing laboratory population of *Drosophila silvestris*. Genetics 116:415–422.

———. 1987*b*. The genetic system, the deme, and the origin of species. Annu. Rev. Genet. 21: 405–423.

Carson, H. L., and A. R. Templeton. 1984. Genetic revolutions in relation to speciation phenomena: the founding of new populations. Annu. Rev. Ecol. Syst. 15:97–131.

Goodnight, C. J. 1987. On the effect of founder events on epistatic genetic variance. Evolution 41: 80–91.

———. 1988. Epistasis and the effect of founder events on the additive genetic variance. Evolution 42:441–454.

Gottlieb, L. D. 1973. Genetic differentiation, sympatric speciation and the origin of a diploid species of *Stephanomeria*. Am. J. Bot. 60:545–553.

Lewis, H., and M. R. Roberts. 1956. The origin of *Clarkia lingulata*. Evolution 10:126–138.

Mayr, E. 1954. Change of genetic environment and evolution. Pages 157–180 in J. Huxley, A. C. Hardy, and E. B. Ford, eds. Evolution as a process. Allen & Unwin, London.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

Powell, J. F. 1978. The founder-flush speciation theory: an experimental approach. Evolution 32: 465–474.

Ringo, J., D. Wood, R. Rockwell, and H. Dowse. 1985. An experiment testing two hypotheses of speciation. Am. Nat. 126:642–661.

Templeton, A. R. 1980. The theory of speciation *via* the founder principle. Genetics 94:1011–1038.

HAMPTON L. CARSON
ROBERT G. WISOTZKEY

DEPARTMENT OF GENETICS
UNIVERSITY OF HAWAII
HONOLULU, HAWAII 96822

Submitted September 12, 1988; Accepted January 4, 1989