experiments and the univariate mapping allows not only the determination of the best reaction conditions but also the specification of tolerances (*1*) within which the factors must be held to achieve this high yield. In particular, the sensitivity of reaction yield to T_2 suggests to us the possibility that other reactions might also exhibit a marked temperature optimum which does not necessarily coincide with the boiling point of the solvent (a choice commonly employed for reasons of convenience). A more detailed study of this and other reactions (including catalytic systems), investigating the effects of more than two factors, is in progress.

WALTER K. DEAN
KATHARINE J. HEALD
Department of Chemistry,
Emory University,
Atlanta, Georgia 30322
STANLEY N. DEMING
Department of Chemistry,
University of Houston,
Houston, Texas 77004

References and Notes

1. A. L. Wilson, *Talanta* **17**, 21 (1970).
2. M. Friedman and L. J. Savage, in *Techniques of Statistical Analysis*, C. Eisenhart, M. W. Hastay, W. A. Wallis, Eds. (McGraw-Hill, New York, 1947), chap. 13.
3. J. J. Carroll, N. Smith, A. L. Babson, *Biochem. Med.* **4**, 171 (1970); C. C. Allain, L. S. Poon, C. S. G. Chan, W. Richmond, P. C. Fu, *Clin. Chem.* **20**, 470 (1974).
4. G. E. P. Box, *Biometrics* **10**, 16 (1954).
5. S. L. Morgan and S. N. Deming, *Anal. Chem.* **46**, 1170 (1974).
6. G. E. P. Box and N. R. Draper, *Evolutionary Operation* (Wiley, New York, 1969).
7. A simplex is a geometric figure defined by a number of points ($n + 1$) equal to one more than the number of dimensions (n) of the factor space. This term does not have the same meaning as the term "simplex" used in linear programming [see, for example, G. B. Dantzig, *Linear Programming and Extensions* (Princeton Univ. Press, Princeton, N.J., 1963)].
8. W. Spendley, G. R. Hext, F. R. Himsworth, *Technometrics* **4**, 441 (1962).
9. J. A. Nelder and R. Mead, *Comput. J.* 7, 308 (1965).
10. Given $n + 1$ simplex vertices represented as n-dimensional vectors $\mathbf{W}, \mathbf{N}, \ldots, \mathbf{B}$ ranked in order of response S_W (worst), $S_N, \ldots, S_B$ (best) and centroid $\bar{\mathbf{P}} = \frac{1}{n}(\mathbf{N} + \ldots + \mathbf{B})$, eliminate $\mathbf{W}$ and evaluate the response S_R at $\mathbf{R} = \bar{\mathbf{P}} + (\bar{\mathbf{P}} - \mathbf{W})$. If $S_N \leq S_R \leq S_B$, retain $\mathbf{R}$. If $S_R > S_B$, evaluate S_E at $\mathbf{E} = \bar{\mathbf{P}} + 2(\bar{\mathbf{P}} - \mathbf{W})$: if $S_E > S_B$, retain $\mathbf{E}$; if $S_E \leq S_B$, retain $\mathbf{R}$. If $S_R < S_N$: if $S_R \geq S_W$, evaluate S_{C_r} at and retain $\mathbf{C}_r = \mathbf{P} + \frac{1}{2}(\bar{\mathbf{P}} - \mathbf{W})$; if $S_R < S_W$, evaluate S_{C_w} at and retain $\mathbf{C}_w = \bar{\mathbf{P}} - \frac{1}{2}(\bar{\mathbf{P}} - \mathbf{W})$. If S_{C_r} or $S_{C_w} < S_N$, set $S_W = S_N$, $\mathbf{W} = \mathbf{N}$, $S_N = S_{C_r}$ or S_{C_w}, and $\mathbf{N} = \mathbf{C}_r$ or $\mathbf{C}_w$. See C. W. Lowe [*Trans. Inst. Chem. Eng.* **42**, T334 (1964)] for an example of a calculation work sheet.
11. S. N. Deming and S. L. Morgan, *Anal. Chem.* **45**, 278A (1973).
12. W. K. Dean and P. M. Treichel, *J. Organometal. Chem.* **66**, 87 (1974).
13. Reactions were carried out as follows: 15 ml of THF and 4.40 ml of a 0.50*M* solution of C_5H_5Na in THF were added to 2.00 mmole (0.528 g) of $Mo(CO)_6$. The mixture was refluxed (65°C) for t_1 and then cooled to T_2; 4.40 ml of 0.50*M* $ClCSN(CH_3)_2$ in THF were then added, and the mixture was stirred for 22 hours in a water bath held at T_2. The reaction yield was determined by adding 100 ml of THF and a weighed sample of benzonitrile (about 3.0 g) to the reaction mixture; the infrared spectrum of the solution was determined on a spectrophotometer (Perkin-Elmer model 467), and the height of the absorption maximum of the product (1841 cm^{-1}) was compared to that of benzonitrile (2245 cm^{-1}).
14. This research was supported by a university research grant from the Emory University Research Fund and by the National Science Foundation (grant GP-32911).

14 April 1975

Drosophila Hybrids in Nature: Proof of Gene Exchange Between Sympatric Species

Abstract. *Genetic studies of two closely related endemic Hawaiian species show that in one area of sympatry about 2 percent of the naturally occurring individuals are hybrids. More than 20 times this many would be expected if the population consisted of a single panmictic unit. Despite hybridization, natural selection appears to maintain the essential integrity of each separate gene pool.*

In the Kahuku area, near the south end of the island of Hawaii, natural interspecific hybridization has been detected between a pair of sympatric endemic species. This circumstance is very unusual for the genus, the other species of which tend to have isolated gene pools which coexist in nature without evidence of gene exchange (*1*). Nevertheless, laboratory crosses between sympatric species are frequently possible and sometimes one or both sexes of F_1 hybrids are fertile (*2*). Accordingly, if reproductive isolation became weak, the potential for gene exchange would exist.

In an extensive literature on *Drosophila* species extending over 50 years, only two cases suggest the possibility of introgression in nature. In both, laboratory crosses give fertile F_1 females and sterile F_1 males. In *D. pseudoobscura* and *D. persimilis*, close genetic scrutiny of the progeny of 27,099 wild-caught flies from sympatric areas yielded four cases of F_1 hybridization (*3*). In each case, a wild female of one species had mated with a male of the other species. Considerable doubt exists, however, as to whether the interspecific matings observed actually took place in nature; since the wild flies were not separated at capture, the interspecific mating could have occurred in the collecting vials on the way to the laboratory. Only a single instance of a backcross hybrid was detected. In *D. metzii* and *D. pellewae*, introgression in nature may be strongly inferred from a face color polymorphism, but critical genetic proof of hybridization in nature is lacking (*4*). We report here a case of hybridization in which one F_1 and three backcross hybrid individuals have been unequivocally recognized in nature.

Drosophila setosimentum and *D. ochrobasis* are near-sibling species endemic to the island of Hawaii (*5*). *Drosophila setosimentum* occurs in rain forests between 600 and 1600 m altitude. At five widely separated locations, all above 1000 m, it is accompanied by *D. ochrobasis*. Both species are rare and local, being largely confined to kipukas (islands of vegetation isolated in the midst of newer lava flows) or collapsed lava tubes where their rare host plants grow. Although no reliable means exists for the separation of females on morphological grounds, males of the two species are easily distinguished by a secondary sexual difference in wing pattern.

Wild-caught females were separated from males at capture. In the laboratory, each female was placed in a separate culture vial and allowed to produce offspring. Such a culture (an isofemale line) usually yields F_1 males conforming exclusively to either one species or the other. From each isoline, seven (sometimes fewer) F_1 larvae were taken, and an acetoorcein squash preparation of the salivary gland chromosomes from each was prepared. From each smear the banding order of all five major chromosome arms (X, 2, 3, 4, and 5) was read directly along the full length of the chromosome, with the use of spaced landmark areas to confirm the sequences. The banding orders of all paired homologs were thereby determined. Inverted sections were read in either homozygous or heterozygous state. The sequence of the hemizygous X in each male larva was also determined.

These cytological data permit inferences about the state of the natural population from which the wild flies were drawn. Except in the Kahuku Ranch population, to be described later, all the F_1 test larvae (195 female and 145 male larvae) examined from 56 wild *D. ochrobasis* females have shown a uniform sequence in chromosome X (*ochrobasis* standard X). In addition, all larvae are homozygous for a chromosome 2 inversion (2k), and all lack an inversion (2l) near the opposite end of the same chromosome. This latter inversion is fixed in all but a North Kona, Hawaii, population of *D. setosimentum*. In addition, the commonest gene order of chromosome 4 of *D. ochrobasis* differs from the standard 4th chromosome of *D. setosimentum* by six inversions. These are spread in a roughly tandem manner over the length of the chromosome.

Similarly, 154 female and 164 male test larvae have been recognized from sympatric areas as the progeny of 54 wild *D. setosimentum* flies. In allopatric areas, 221

D. setosimentum were also examined (1062 test larvae). Collections from Kahuku Ranch are excluded from these counts.

Accordingly, from two to nine chromosomal markers serve to distinguish each individual of each species. Their homozygosity and their distribution in the genome is such that three of the five major chromosome pairs are well marked. The lack of heterozygotes in samples of the size reported above means that the probability of incorrect diagnosis of species and of failing to recognize F_1 and backcross hybrids between the species is very small. Had we been dealing with one population at Hardy-Weinberg equilibrium, 55 heterozygotes at any one of the autosomal markers would have been expected.

In the laboratory, hybrids between the species are fairly easily made. The resultant chromosomal heterozygotes are those that would be expected from the readings of the two sets of homozygous states. Thus, the *D. ochrobasis* X chromosome in female hybrids is observed in heterozygous state with one of four alternate gene orders known to be polymorphic within *D. setosimentum*. In addition, the expected two-inversion difference ($2k\ 2l^{+}/2k^{+}\ 2l$) appears in chromosome 2 and the expected six-inversion heterozygote appears in chromosome 4. Further, *D. ochrobasis* adults have a distinctive fixed electrophoretic difference relative to *D. setosimentum*. This may be observed at a β-naphthyl acetate esterase (Est-1) locus (*6*).

Cytological or electrophoretic assays (or both) have been carried out on 180 wild specimens collected from the Kahuku Ranch, Kau District, island of Hawaii. Polytene chromosomes of seven F_1 larvae from each isofemale line were scored, and each larval corpse was subjected to electrophoresis. Parallel cytological and electrophoretic data was thus provided for a total of 744 test larvae. Most wild-caught males were subjected to electrophoresis without cytological examination, but some were analyzed by crossing to laboratory virgins from a monomorphic stock and subjected to electrophoresis after F_1 larvae had been produced by their mates.

Four exceptional individuals were found. The first was a wild female that transmitted a *D. setosimentum* X chromosome to her sons but showed a *D. ochrobasis–D. setosimentum* heterozygous X in her female progeny. Autosomes 2 and 4 of both sexes showed the hybrid condition in all test larvae. As the female was isolated at capture, this is an unequivocal case (based on heterozygosity for four independent chromosomal markers) of a wild *D. setosimentum* female which had mated with a *D. ochrobasis* male in nature.

The second case was that of a larva collected from a natural oviposition site, a fermenting branch of the plant *Clermontia*. Fourteen third instar larvae from the branch were smeared and the tissues of the same larvae were subjected to electrophoresis. In addition, eight emerging adults from the same branch were subjected to electrophoresis. Of these 22 specimens, 20 were *D. ochrobasis*, one was *D. setosimentum*, and one was a female backcross hybrid. The latter displayed the double polymorphism in chromosome 2 but was homokaryotypic for the *D. ochrobasis* X and 4th chromosomes. Finally, two wild male flies having *D. setosimentum* wing patterns, out of 40 electrophoretically tested but not studied cytologically, proved to be heterozygous for the highly distinctive "null" allele of *D. ochrobasis* at the Est-1 locus.

The foregoing data constitute proof that gene exchange actively occurs between contemporary populations of these species in this locality. Of 180 specimens studied, 4 (2.2 percent) were hybrids. If the population were at Hardy-Weinberg equilibrium, 89 heterozygotes, more than 20 times the observed number, would be expected at the autosomal 2k inversion region alone. The sites at which collections were made are in almost wholly undisturbed rain forest, so that one is not tempted to invoke the idea that man has "hybridized the habitat" (*7*) and thus provided unusual ecological conditions which might have been conducive to the hybridization process or to the biological success of its genetic products.

Discovery of F_1 and backcross hybrids is of particular interest in view of Carson and Johnson's suggestion (*5*) that these species have hybridized in the past, as evidenced by the existence of a series of peculiar kinked homologs ("complex chromosomes") which have been found segregating within most sympatric populations of both species. In gene order these kinked chromosomes resemble certain chromosomes of the other species; this appears to have resulted from reciprocal exchange occurring in past generations. Why they have become complexly kinked is not known.

Geographical speciation theory can be invoked to explain the origin of these species in an ecosystem that is continually being fractionated by lava flows (*8*). Nevertheless, the conventionally isolated gene pools of each are not now closed to exchange with the other at places where they come in contact. As in many plants, introgression (*9*) is occurring and apparently has occurred in the past. Despite this, however, the gene pools of the two species give no evidence of flowing together and undergoing free genetic recombination. Accordingly, we hold that the hybridization circumstance is a manifestation of recent (in the geological sense) divergence between these species. Although each species has apparently evolved a recognizable and integrated gene pool of its own, each remains open to the acquisition of small amounts of genetic variability from the other.

This leaky boundary merely represents a stage in what may be a rather common pattern of allopatric speciation in general. Two separated populations may come back into contact after each has virtually completed the integration of its new gene pool. If there is only weak reproductive isolation full recombination might occur. In this, as in many other cases in plants and animals, the reciprocal flow of genes appears to be insignificant probably because of natural selection working against inferior F_2 and backcross combinations. The extent of such hybrid zones between closely related species varies greatly; thus the broad zones found in some plants differ greatly from the small zone found in this *Drosophila* case.

A recent theory suggests that a species, as distinguished from an infraspecific population, is characterized by a unique integrated internal system of genetic balance (*10*). So long as closed and balanced genetic systems are not destroyed by recombination, populations can coexist even in the presence of considerable interbreeding. The case we discuss conforms to this view. This is to be expected on a large isolated tropical island which is geologically new and which has rain forests which continue to be dissected by recent lava flows.

H. L. CARSON
Department of Genetics,
University of Hawaii, Honolulu 96822
P. S. NAIR
Department of Biology, Southern
Illinois University, Edwardsville 62025
F. M. SENE
Department of Biology CP11461,
University of São Paulo,
São Paulo, 05421 Brazil

References and Notes

1. J. T. Patterson and W. S. Stone, *Evolution in the Genus* Drosophila (Macmillan, New York, 1952).
2. E. Craddock, *Evolution* **28**, 593 (19 H. Carson, *ibid.* **8**, 148 (1954).
3. Th. Dobzhansky, *Am. Nat.* **107**, 312 (1973).
4. S. Pipkin, *Evolution* **22**, 140 (1968).

H. Carson and W. Johnson, *ibid.* **29**, 11 (1975).

6. P. Nair, F. Sene, H. Carson, *Genetics* **80**, s60 (1975).
7. E. Anderson, *Evolution* **2**, 1 (1948).
8. H. Carson, *Stadler Symposia* **3**, 51 (1971).
9. V. Grant, *Plant Speciation* (Columbia Univ. Press, New York, 1971).
10. H. Carson, *Am. Nat.* **109**, 83 (1975).
11. Supported by NSF grants GB27586 and GB29288 to the University of Hawaii and by Office of Research and Projects, Southern Illinois University. F.M.S. is postdoctoral fellow of Fund. Amparo a Pesquisa do Est, S. Paulo, Brasil. We thank K. Y. Kaneshiro and L. T. Teramoto for help.

1 May 1975; revised 3 June 1975