

- Electroencephalogr. Clin. Neurophysiol.* **38**, 13 (1975).
7. R. Galambos, P. Benson, T. S. Smith, C. Schulman-Galambos, H. Osser, *ibid.* **39**, 279 (1975).
  8. E. Callaway, in *Habituation: Physiological Substrates*, H. V. S. Peeke and M. J. Herz, Eds. (Academic Press, New York, 1973), pp. 153-174.
  9. T. Allison, *Electroencephalogr. Clin. Neurophysiol.* **14**, 331 (1962); H. Davis, T. Mast, N. Yoshie, S. Zerlin, *ibid.* **21**, 105 (1966).
  10. Subjects were considered right-handed if they had a right-sided preference for batting, writing, and kicking and if all immediate family members were right-handed.
  11. Position C<sub>2</sub> is an active electrode site that elicits the clearest response to auditory stimuli [W. R. Goff, Y. Matsumiya, T. Allison, G. D. Goff, in *Average Evoked Potentials: Methods, Results and Evaluations*, E. Donchin and D. B. Lindsay, Eds. (National Aeronautics and Space Administration, Washington, D.C., 1969), pp. 95-141]. Studies using the AEP that were not concerned with lateralization effects have typically recorded between C<sub>2</sub> and one or more ear references. Pilot experiments in our laboratory, using different electrode sites, including a reference with the ears linked, indicated that the temporal-to-C<sub>2</sub> placement produced the clearest lateralized responses to the different task requirements and allowed the averaging of fewer responses to obtain scoreable AEP peaks. Even though C<sub>2</sub> probably produces a larger evoked response than the lateral sites used in this study, it is possible that for this reason the response amplitude is potentially more variable than it is with other electrode arrays; it would thus be more sensitive to the lateralizing effects of the tasks. Further, since C<sub>2</sub> is common to both temporal placements, differences in electrical activity at the temporal recording sites would still be discernible from the bipolar response.
  12. Grass Gold-plated electrodes (Grass) were used to record the AEP's between T<sub>4</sub>-C<sub>2</sub> and T<sub>2</sub>-C<sub>2</sub> (according to the 10-20 International System), with a ground electrode located on the forehead. Electrode impedance was maintained below 5000 ohms and was checked and recorded at the beginning of each condition. Two amplifier channels of a polygraph (Grass model 78) with a bandpass of 0.3 to 300 hertz and a sensitivity of 7.5  $\mu$ V/mm were used to record the EEG. The AEP's for tone 1 and tone 2 were summed separately on a signal averager (Nicolet 1072) and printed out on an X-Y plotter (Hewlett-Packard 7004b). Two calibrators (Bioelectric CA5) in series with the subject's scalp were used to place a 10- $\mu$ V, 20-msec calibration signal on the left- and right-hemisphere AEP's.
  13. All of the material for each condition, including the instructions, was prerecorded on audiotape and presented to subjects through stereophonic headphones while they were seated in a reclining chair in a sound-attenuated, electrically shielded room. A mixer (Sony MX-14) was used to control intensity ratios between tone pips and the task material presented in each of the three conditions. Tone pips were produced by a waveform generator (Interstate Electronics Corporation). Tone-pip intensity as measured by an impulse precision sound level meter (Brüel and Kjaer) was approximately 84 db sound pressure level, according to the C-weighting network specified by International Electrotechnical Commission recommendation 179. The average intensity of the white noise, verbal passages, and musical selections was 76 db  $\pm$  5 db. Stimulus intervals, stimulus duration, and triggering of the averager and calibration equipment was controlled by a Siliconix System.
  14. Passages were taken from the reading test section of E. C. Gruber, *The Complete Study Guide for Scoring High: Graduate Record Examination* (Arco, New York, 1970).
  15. A. L. Edwards, *Experimental Design in Psychological Research* (Holt, Rinehart & Winston, New York, 1960), pp. 136-140.
  16. The general AEP amplitude differences between baseline, verbal, and music conditions were probably due to subjective differences in the intrusiveness of the tone pips on incoming white noise, verbal, and musical stimuli. Most subjects, when questioned at the end of the experimental session, indicated that during the music condition, the tone pips were less intrusive than they were during the verbal condition; they were most intrusive during the baseline condition. Thus, it appears that a contextual effect between the tone pips, and ongoing white noise, verbal, and musical stimuli produced AEP's of generally higher amplitude during the baseline condition, and higher-amplitude AEP's for the verbal compared with the music condition.
  17. M. Kinsbourne, in *Attention and Performance IV*, S. Kornblum, Ed. (Academic Press, New York, 1973), pp. 239-256.
  18. D. Galin, *Arch. Gen. Psychiatry* **31**, 572 (1974).
  19. We thank M. Andrew for his technical assistance with the audio stimuli and Drs. A. Friedman, J. Campos, and S. Hoffman for their helpful comments. Supported in part by NIH grant MH 28513 to D.W.S.

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## Sympatric Speciation Based on Allelic Changes at Three Loci: Evidence from Natural Populations in Two Habitats

**Abstract.** *Allelic changes at three loci largely explain Chrysopa downesi's sympatric speciation from a Chrysopa carnea-like ancestor. Disruptive selection first produced a stable polymorphism based on a single pair of alleles that adapted individuals to two habitats, and second, it established seasonal asynchrony in reproduction through allelic substitutions at two loci.*

A central problem in evolutionary biology concerns the amount and type of genetic change and the procession of events that produce independent evolutionary units—species. Genetic divergence of geographically isolated populations (allopatric speciation) is generally accepted as the primary mode of speciation in bisexual animals (1). In contrast, the concept of speciation through the action of disruptive selection on an interbreeding population (sympatric speciation) remains an area of considerable controversy. In the selection experiments around which the modern theory of sympatric speciation was built, the

physiological and genetic basis for the reproductive isolation produced in the laboratory is unknown, and the artificial forces of disruptive selection are not related to field conditions (2). Therefore, these experiments have provided relatively limited insight into the question of sympatric speciation in natural populations.

As a testable hypothesis, Maynard Smith (3) offered a general theoretical model for sympatric speciation through disruptive selection. This model's attractiveness resides in its simplicity—a few, simple genetic changes underlie the proposed process of speciation. Until now,

the most convincing field and laboratory evidence for this model has come from monophagous insects in which simple genetic changes produce divergent host races that lead to new species (4). Indeed, it has been suggested that sympatric speciation through disruptive selection is restricted to host-specific, phytophagous or parasitic animals (5). In contrast, we report experimental evidence herein that disruptive selection can act through habitat differences in the speciation of non-host-specific animals. In our example, a single gene difference underlies a divergence in habitat association, and allelic substitutions at two loci underlie the subsequent evolution of an effective reproductive barrier. This study illustrates that Maynard Smith's theoretical model for sympatric speciation, rather than being restricted to monophagous or parasitic species, has broad application among bisexual animals.

Our experimental animals were the sibling species *Chrysopa carnea* Stephens and *Chrysopa downesi* Banks (Insecta: Neuroptera: Chrysopidae), which occur sympatrically in northeastern United States. They share many important features in their biology; for example, the larvae of both species prey on a variety of soft-bodied arthropods, and the adults feed on honeydew and pollen and have similar dietary requirements for reproduction (6). Under laboratory conditions these species hybridize and produce fully viable and fertile F<sub>1</sub> and F<sub>2</sub> offspring. However, in nature the two species are reproductively isolated through differences in habitats and in seasonal periods of reproduction (7).

The species *C. carnea*, which is multivoltine (producing several generations each summer), occurs mainly in grassy areas and meadows during its annual reproductive period (late spring to the end of summer). At this time the pale green adults are cryptically colored against a background of light green foliage. At the end of summer, when reproduction ceases, the adults enter reproductive diapause and move to the senescent foliage of deciduous trees. This movement by the *C. carnea* adults is accompanied by a change in color, from light green to reddish-brown, thus maintaining the adults' camouflage in their overwintering site. In contrast to *C. carnea*, *C. downesi* is a univoltine, early-spring breeder, and it is restricted to conifers throughout the year. The very dark green color of *C. downesi* adults camouflages them in their coniferous habitat during both reproduction and diapause.

In hybridization tests, individuals homozygous for the semidominant autosomal allele, *G*, exhibit the dark-green phenotype of *C. downesi* adults, whereas the recessive *g* alleles produce the light-green phenotype. In *Gg* heterozygotes color is intermediate, and the expressivity of the *G* allele is modified by polygenes (8). Furthermore, single allele differences at two unlinked autosomal loci form the basis for the seasonal asynchrony in the *C. carnea* and *C. downesi* reproductive cycles—with *C. downesi*'s univoltinism the result of recessive alleles ( $d_1d_1$  and  $d_2d_2$ ) at both loci (9).

We propose that *C. downesi*'s speciation from a *C. carnea*-like ancestor (10) occurred in two steps, similar to those proposed by Maynard Smith in his model for sympatric speciation. The first step included the establishment of a stable polymorphism, based on a single pair of alleles, through the action of disruptive selection in a two-habitat situation. The second step, the evolution of reproductive barriers between the forms occupying the two habitats, involved the establishment of the recessive alleles controlling *C. downesi*'s univoltinism.

We propose that the first step in *C. downesi*'s evolution was the selection for homozygosity of gene *G*, the autosomal, semidominant allele that produces the dark-green adult color. This change allowed the ancestral population of *C. downesi* to occupy successfully a coniferous habitat that previously had been unfavorable; against coniferous foliage, the dark-green adults appear well concealed, presumably from vertebrate predators (11). In contrast, the dark-green color was deleterious in the original habitat. The heterozygotes with their intermediate coloration, though superior to either form in the "wrong" habitat, were at a competitive disadvantage to the homozygotes in their respective habitats. Thus, the population was subjected to disruptive selection which produced and maintained a stable, two-allele polymorphism involving pale- and dark-green color forms—each restricted and adapted to its own habitat. At this point in the evolution of *C. downesi*, the polymorphism would have been maintained by disruptive selection even if the two color forms mated at random. There was no genetically controlled preferential mating between the two forms; however, the frequency of interform pairings was probably low because the adults of each form mainly occurred in their own habitat.

With the establishment of the stable, two-habitat polymorphism, each of the

morphs was subject to the selective pressures characteristic of its own habitat. In the coniferous form, selection pressure resulting from the annual occurrence of food and competitors favored the restriction of reproduction to early spring (12). Thus, on the one hand, early-spring reproduction synchronized the annual distribution of larvae and adults with the occurrence of favorable conditions on conifers; and, on the other hand, early-spring reproduction acted as an effective barrier to interbreeding between the forms because of its asynchrony with reproduction by the nonconiferous form (7).

The seasonally asynchronous reproduction by *C. carnea* and *C. downesi* results from single allele differences at each of two autosomal loci (9). These alleles produce differential responses to photoperiod which, in turn, are responsible for the seasonal occurrence of reproduction in the two species (7-9). Therefore, the recessive  $d_1$  and  $d_2$  alleles that underlie *C. downesi*'s restricted univoltine seasonal cycle also act as assortative mating genes. We propose that the selection for these alleles and their replacement of their dominant  $D_1$  and  $D_2$  counterparts in the coniferous form, constituted the second step in *C. downesi*'s speciation (13). The  $d_1$  and  $d_2$  alleles were probably strongly selected against in the nonconiferous form because a high rate of reproduction was advantageous in the original habitat (grassy areas and meadows), and even one recessive  $d_1$  or  $d_2$  allele exerts a negative effect on the reproductive rate (8). Thus, the  $d_1$ ,  $D_1$ ,  $d_2$ , and  $D_2$  alleles were subjected to disruptive selection in relation to habitat. The dominant  $D_1$  and  $D_2$  alleles were selected preferentially in the original nonconiferous form, and the recessive  $d_1$  and  $d_2$  alleles replaced them in the coniferous form. With the completion of this process, the seasonal cycles of the two forms became asynchronous, interbreeding between the forms virtually ceased, and *C. downesi* was established as a species.

These events and our interpretation of them do not imply that *C. carnea* and *C. downesi* now differ by only three genes; on the contrary, we have identified several other areas of genetically based divergence between the species (7, 8). Presumably, this divergence resulted from the evolution of adaptive features that further increased the fitness of the two species in their respective habitats.

To summarize, the essential features of our study are twofold. (i) As few as three gene substitutions can result in

speciation in which the resulting species have distinct habitat differences and efficient premating barriers to hybridization. (ii) Such gene substitutions can occur, in the absence of geographic isolation, as a result of disruptive selection on an interbreeding population. We expect that this mode of speciation is not uncommon among animals with marked habitat differences, and elsewhere (8) we present a genetic model for speciation through habitat diversification and seasonal isolation.

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#### References and Notes

1. E. Mayr, *Animal Species and Evolution* (Belknap, Cambridge, Mass., 1963); G. L. Stebbins, *Processes of Organic Evolution* (Prentice-Hall, Englewood Cliffs, N.J., 1971); Th. Dobzhansky, *Genetics of the Evolutionary Process* (Columbia Univ. Press, New York, 1970); F. J. Ayala, *Evol. Biol.* 8, 1 (1975); G. L. Bush, *Annu. Rev. Ecol. Syst.* 6, 339 (1975).
2. See B. Wallace [Topics in Population Genetics (Norton, New York, 1968), pp. 397-401] and J. M. Thoday and J. B. Gibson [*Am. Nat.* 104, 219 (1970)] for critical reviews of disruptive selection experiments as they pertain to sympatric speciation.
3. J. Maynard Smith, *Am. Nat.* 100, 637 (1966).
4. G. L. Bush, *Evolution* 23, 237 (1969); M. D. Huettel and G. L. Bush, *Entomol. Exp. Appl.* 15, 465 (1972); see also G. Knerer and C. E. Atwood, *Science* 179, 1090 (1973); P. A. Phillips and M. M. Barnes, *Ann. Entomol. Soc. Am.* 68, 1053 (1975); S. Khasimuddin and P. DeBach, *Entomophaga* 21, 113 (1976).
5. G. L. Bush, in *Evolutionary Strategies of Parasitic Insects and Mites*, P. W. Price, Ed. (Plenum, New York, 1975), pp. 187-206; *Annu. Rev. Ecol. Syst.* 6, 339 (1975).
6. M. J. Tauber and C. A. Tauber, *Can. Entomol.* 106, 921 (1974).
7. ———, *Can. J. Zool.* 54, 260 (1976).
8. ———, *Nature (London)*, in press.
9. C. A. Tauber, M. J. Tauber, J. R. Nechols, *Science* 197, 592 (1977).
10. Evidence for *C. carnea*'s earlier derivation than *C. downesi*'s lies in its broad geographic distribution (over most of the Holarctic region) and in its generalized biological characteristics; for example, *C. carnea* exhibits a broad plant association and multivoltinism. In contrast, *C. downesi* has a restricted geographic distribution (more northern regions of North America), a narrow habitat range, and a univoltine life cycle.
11. We have observed that conifer-inhabiting chrysoiid species, in general, are either cryptically (dark-green) colored as in *C. harrisii* Banks and *C. downesi*, or they produce a malodorous secretion as in *Meleoma emuncta* (Fitch) that is offensive to predators [compare M. S. Blum, J. B. Wallace, H. M. Fales, *Insect Biochem.* 3, 353 (1973)].
12. We have evidence (M. J. Tauber and C. A. Tauber, in preparation) that competition for prey among conifer-inhabiting arthropod predators increases markedly during summer. *Chrysopa downesi*'s early-spring reproduction results in the occurrence of its predaceous larvae during late spring, before many of its competitors become active.
13. We do not have evidence that shows which of the two genes ( $d_1$  or  $d_2$ ) first replaced its dominant counterpart; however, we do have evidence (8) suggesting that the alleles at each locus can act independently to restrict *C. downesi*'s reproductive period. Therefore, the mutation and selection of  $d_1$  and  $d_2$  need not have occurred simultaneously.
14. We thank B. Wallace, Cornell University, and J. T. Doyen, University of California, Berkeley, for their comments on our work.

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