

## Sex Determiners and Speciation in the Genus *Chironomus*<sup>1</sup>

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**ABSTRACT:** In a group of closely related Australian *Chironomus* species, the position of the sex-determining region is variable, not only in different species (or forms) but, in at least one case, also within the same form. This has raised a number of questions regarding the nature of sex determination and the relevance of variable sex determiners to speciation: (1) Is the sex-determiner location altered by mutation at different steps in a genetic pathway or by translocation (e.g., as a transposable element)? (2) Do polymorphisms for sex-determiner location exist, or do the apparent polymorphisms result from the existence of cryptic species? (3) Are changes in the location of the functional sex determiner a major component of speciation in this group? This paper considers mainly the third question. A model of speciation involving the sex determiner, compatible with either allopatry or sympatry, is proposed. Comparisons are made with other groups, both invertebrate and vertebrate, which appear to have a similar variable sex-determiner location.

SEX DETERMINATION IN THE GENUS *Chironomus* is male heterogametic, the functional sex determiner being a single gene, or at most a small group of genes. This gene(s) acts as a dominant male determiner, such that in its absence the individual will develop as a female but the presence of the male determiner switches development to that of a male (Martin and Lee 1984a). The mechanism by which the sex-determiner gene(s) exerts its effect is presently unknown. In most natural populations of *Chironomus* there is no cytological indication of the location of the sex determiner, although in some species the general location may be indicated by sex-linked inheritance of inversions or heterochromatic bands (Martin et al. 1980).

The basic karyotype in *Chironomus* is four pairs of chromosomes, or four polytene elements, of which three are metacentric and one acrocentric (Bauer 1945). The seven chromosome arms, identified as A-G, are combined differently in different groups of

species (Keyl 1962). In the initial identification of sex determiners in *Chironomus*, Beermann (1955) reported a different location of the sex determiner, one in arm B and the other in arm F, in each of two populations of *C. tentans*. Subsequent work has indicated that the site of the sex determiner may differ between species and also within the same species. Up to four different locations have been identified in what appears to be a single species (Martin and Lee 1984b), but any given individual appears to have a single sex-determiner location. Bull (1983) suggests that this may be consistent with his multiple-factor system 3.A.1.

Sex-determiner locations have been found on all chromosome arms except arm E (Martin and Lee 1984b), with the reason for their absence from arm E being unknown. Exact mapping of the sites is difficult in general because of the lack of appropriate genetic markers, although it has been achieved in a few cases (Martin and Lee 1984b). Therefore, it is difficult to determine whether sex determiners on the same arm are at the same chromosomal location, particularly where there have been chromosomal rearrangements. Arms A, F, and G appear to contain at least two sex-determiner locations in each arm (Martin 1981, Martin and Lee 1984b).

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Two hypotheses have been advanced to explain the occurrence of multiple sex-determiner locations: (1) A biochemical pathway is involved, the site of the sex determiner reflecting the point in the pathway at which a mutation resulting in a change in sex determination has occurred (Bedo 1975, Martin and Lee 1984b). (2) The sex-determining loci are moved by translocations (e.g., Mainx 1964) or by association with a transposable element (Green 1980, Rothfels and Nambiar 1981, Martin and Lee 1984b). These two hypotheses lead to different predictions. If multiple locations are due to mutations in a pathway, there will be few defined locations. Also, because the functional sex determiner could occur only at progressively earlier steps in the biochemical pathway, mutations in the latter part of the pathway could be present but would not be apparent unless separated from the earlier mutation by recombination. The presence of only one sex determiner in any individual would argue against this explanation. Mainx (1964) found that the sex determiners in *Megaselia scalaris* were at the ends of the chromosomes, and suggested that they may be moved by small terminal translocations. The identification of sex determiners near the centromeres and in interstitial positions in *Chironomus* indicates that terminal rearrangements cannot be involved, but may be explained by assuming that the sex determiners are associated with a transposable element. If multiple locations are due to association with a transposable element, there should be a significant number of locations, few of which would be common among species, unless of common descent. The concept of sex determiners associated with a transposable element is also consistent with the observation that there is no cytological evidence of translocation associated with any known changes of the sex-determiner locations. A test of these alternative hypotheses was suggested by Martin and Lee (1984b).

The genus *Chironomus* is also characterized by the existence of large complexes of sibling species, e.g., the *C. thummi* complex in Europe, the *C. decorus* complex in North America, and the *C. alternans* complex in Australia. Relatively little evidence exists

for the locations of sex determiners in either of the first two mentioned complexes, but in the *C. alternans* complex and in the other related *Chironomus* species of south eastern Australia, the location of the sex determiner commonly changes between species (Martin and Lee 1984b). However, change in the sex-determiner location does not always occur between species as, for example, there appears to be a group of species central to this phylogenetic group with a sex determiner on the CD chromosome, although it is not certain that it is in the same place in each species (Martin and Lee 1984b). On the other hand, there are a number of instances in which the location of the sex determiner has definitely changed between species. Perhaps the most obvious is in the *C. australis* complex, in which the derived species *C. duplex* has the sex determiner on arm B, while the species from which it appears to have been derived has the sex determiner on the CD chromosome, and another close relative has the sex determiner on arm A (Martin et al. 1980). However, most interesting are the forms of *C. oppositus*, most of which have the sex determiner in a different specific location (Martin and Lee 1984b). Although the status of these forms has not been defined, the rarity of hybrids in the wild suggests that they are effectively species. One of these forms, form *whitei*, appears to be polymorphic for four different sex-determiner locations. It has been argued elsewhere (Martin and Lee 1984b) that such a polymorphism is unlikely and that the apparent polymorphism may indicate the presence of further (cryptic) species. Unpublished data suggest that this may be at least a partial explanation, since those samples in which the male has a sex determiner on the CD chromosome are distinguishable by a different combination of inversions from the samples with any of the other three sex-determiner sites. This enhances the concept of *C. oppositus* as a complex of species that differ in the location of the sex determiner.

The variability in the location of the sex determiner in *Chironomus* raises the possibility that the change in position of the sex determiner is related to speciation, perhaps

coincidental but possibly integral to the speciation process in at least some cases. Although this possibility has been raised previously (White 1978, Rothfels and Featherston 1981), no proposed mechanism has been published. However, the polymorphism for sex determiners that exists and the observation that recombination suppressors (e.g., inversions or heterochromatic blocks) tend to accumulate in the vicinity of sex determiners (Bedo 1975, Martin et al. 1980) suggests a possible mechanism. If a species becomes polymorphic for two sex-determiner locations and each lies adjacent to a group of genes with adaptive properties, any restriction of recombination between these adaptive genes and the sex determiner will result in their transmission as a unit, at least in males. This will result in disruptive selection, particularly if the gene combinations assist in the exploitation of different ecological niches. No selective difference is proposed for the sex determiners themselves. This is similar to some of the models analyzed by Bull and Charnov (1977), in which a mutant sex-determiner system is successful if some of the genotypes formed have higher viabilities than an alternative system.

Speciation by this method may occur under either allopatry or sympatry. In allopatry, the alternative sex-determiner locations with their associated genes would be selected in different parts of the geographic range, or one of the combinations might permit the exploitation of a new habitat. In a sympatric situation, speciation of sections of the population adapted to slightly different ecological niches would be assisted if behavioral differences, particularly relating to sexual selection, were involved. In *Chironomus*, such behavioral factors could involve the height at which a mating swarm is formed, the type of swarm marker used, or the time of day at which the swarming occurs (Martin and Lee 1981). Various models of sympatric speciation are applicable, particularly that of Maynard Smith (1966), involving ecological divergence, or the model of Wu (1985), involving sexual selection.

Differences between two of the forms of *C. oppositus*, form *tyleri* and form *connori*,

suggest that both ecological and sexual selection components may be involved, although it is not known whether the divergence was allopatric or sympatric. Larvae of form *tyleri* live in deeper water than those of form *connori*, and both larvae and adults of form *tyleri* are larger (Martin, Lee and Connor 1978, Martin and Lee 1981, Kuvangkadilok 1984). The adults of these forms probably swarm at different heights, since the height at which swarming occurs is normally inversely proportional to size (Gibson 1945). If the females choose to enter a swarm of males of equivalent size, then assortative mating due to sexual selection will occur. This suggests that such a mode of speciation is possible. The available data indicate that many of the related *Chironomus* species considered in this paper are not distributed allopatrically but broadly sympatrically, although they may live in somewhat different niches. Since the relationship between present distributions and those existing at the time of speciation is unknown, we cannot be certain whether allopatric or sympatric speciation has been involved in any particular case. Rothfels and Featherston (1981) suggest that the differentiation of sibling species in *Simulium vittatum* has occurred by sympatric speciation involving the sex determiner. The model is described in an unpublished manuscript.

Sex determination in *Chironomus* resembles systems reported in such diverse organisms as simuliids (Rothfels and Nambiar 1981), houseflies (Franco et al. 1982), some mosquitoes (Baker et al. 1977), phorids (Mainx 1964), frogs (Elinson 1983, Wright and Richards 1983), and some lizards (Moritz 1984), in that the sex of an individual is controlled by a single gene or group of genes that may be translocatable. It is generally agreed that the sex-determining systems of insects and vertebrates have evolved independently (Vorontsov 1973). However, the above list indicates a similarity in the sex-determining mechanisms of the lower Diptera and those of the lower vertebrates, consistent with the view that sex determination may be associated with transposable elements (Hickey 1982, Rose 1983, Jones 1984). Both the Diptera and the vertebrates show

development of differentiated sex chromosomes, particularly in the more highly evolved genera. This suggests that parallel evolution of the sex-determining mechanisms has occurred in these diverse phyla. It also suggests that the model for the involvement of the sex-determining genes in speciation outlined here for *Chironomus* may be applicable to a broader range of species.

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