SEX-LINKED INVERSIONS IN BLACKFLIES (DIPTERA: SIMULIIDAE)

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SUMMARY

The family Simuliidae exhibits a diversity of sex-linked inversions which fall into four major classes. Inversions occur which are found only on the *X* or *Y*, and these may be fixed or polymorphic. *Y*-linkage is clearly commoner amongst such inversions. Other inversions are found which phylogenetic evidence suggests cannot have been sex-linked in ancestral species, but have become so in derived forms. Lastly, inversions are found which occur on both sex chromosomes, but at different frequencies.

1. INTRODUCTION

IN most cases of sex chromosome heteromorphy it is the *Y* chromosome which is the smaller element, and theories proposed to account for this are based on the unique property of the *Y*, that it only ever occurs in the heterozygous state. Thus it alone is able to degenerate by the accumulation of recessive deleterious mutations, such as small deletions and other gene dysfunctions, because these will be masked by the equivalent functional locus on the *X* chromosome (Muller, 1914). Specific numerical models devised to test this hypothesis assume recombination is already suppressed between the *X* and *Y* (Nei, 1970; and Charlesworth, 1978). Thus attention focuses on mechanisms which restrict recombination between sex chromosomes, and structural rearrangement by inversion is one such mechanism.

The Simuliidae is a family of small blood-sucking flies with immature stages restricted to flowing water. Many species of this family have been subject to polytene chromosome analysis and as a result it has been possible to construct extensive phylogenies, sometimes embracing several genera. These studies have also revealed an impressive assortment of inversions linked to the sex chromosomes in one way or another. Such inversions represent a process of sex chromosome differentiation which results in the restriction of recombination between the *X* and *Y*, and thus could lead on to the process of *Y* chromosome degeneration. It is therefore of interest to analyse the behaviour of these inversions, and to this end a classification has been devised into which all the sex-linked inversions known from the Simuliidae can be placed.

Polytene chromosome studies of the Simuliidae also frequently reveal the existence of sibling species and it seems that quite often the karyotypes of such species differ only in their sex chromosomes (Rothfels, 1979). This has led to the suggestion that structural rearrangement of the sex chromosomes may be an integral part, if not the driving force in Blackfly speciation (Bedo, 1977; White, 1978). If this is true the present day behaviour of the sex-linked inversions may allow us some insight into how the process occurred.

2. THE SYSTEM OF CLASSIFICATION

Most blackflies have indistinguishable sex chromosomes, and this is presumably the ancestral condition. However, there are species in most groups which have undergone some form of sex chromosome differentiation, usually by linkage of inversions, and this appears to have arisen *de novo* within each particular species. There is good phylogenetic evidence in only one case for a pair of differentiated sex chromosomes persisting through a speciation event (*Simulium congareenarum* group: Dunbar, 1967).

The classification developed here is based on the exact pattern of linkage of inversions to the sex chromosomes and relies heavily upon the above supposition. For example, if a species is found to possess a unique inversion such that one sequence is linked to the *X*, and the other sequence to the *Y*, then if all other species examined from that group are found to possess only the *X* sequence it is assumed that this is the ancestral arrangement (labelled as such by the suffix ₀ —i.e., *X*₀), whereas the *Y* sequence is the derived, inverted condition (distinguished by the suffix of a positive number, e.g., *Y*₁).

The classification defines four categories. Inversions which occur on the sex chromosome pair but show no difference in frequency between *X* and *Y* are quite common but are not included.

- (1) Inversions which are found fixed on either of the sex chromosomes.
 - (a) Fixed on *Y*, i.e., *X*₀, *Y*₁
 - (b) Fixed on *X*, i.e., *X*₁, *Y*₀
- (2) Inversions which are floating, but only occur on one of the sex chromosomes (i.e., there is a polymorphic *X* or *Y*).
 - (a) Linked to *Y*, i.e., *X*₀, *Y*₀, *Y*₁, *Y*₂, ... *Y*_{*n*}
 - (b) Linked to *X*, i.e., *X*₀, *X*₁, *X*₂, ... *X*_{*n*}, *Y*₀
- (3) Inversion polymorphisms which, according to phylogenetic evidence, were not sex-linked in ancestral species, but have become so in derived species. Standard and inverted sequences may be fixed on either *X* or *Y*. Such inversions I call ancient polymorphisms.
- (4) Inversions at different frequencies on *X* and *Y*.

Table 1 lists the sex-linked inversions according to species. As far as possible the numbering of these inversions follows that of the original author, and is given in brackets after the designation of the category of sex linkage, according to the code described above. For example, *X*₀, *X*₁(2S-4), *Y*₀ would indicate an inversion, numbered as "4" by the original author, in the short arm of chromosome 2 and described as being polymorphic on the *X*, but never being found on the *Y* chromosome.

Rearrangements of the polytene banding sequence which cannot be ascribed to specific inversions are termed indeterminate rearrangements; these and multiple rearrangements are treated as single inversions, unless there is phylogenetic evidence of stepwise derivation. For example, a double inversion of this sort is seen in *S. tuberosum* "FG" and is labelled 2S-F. G, whereas in *S. tuberosum* "AB" there is evidence of stepwise derivation, because one of the inversions occurs independently, and the double inversion is labelled 2S-A + B.

TABLE 1
Sex-linked inversions in blackfly species

Species	Sex-linked inversions	Notes	Authors
<i>Simulium ornaticipes</i> group			
<i>S. ornaticipes</i> "A"	X_0, Y_0, Y_1 (1L-16)		Bedo (1977)
<i>S. ornaticipes</i> "B"	X_0, Y_0, Y_1 (1S-4)—ancient polymorphism		
<i>S. neornaticipes</i> "1"	X_1 (3L-12), Y_1 (3L-13), Y_2 (3L-13 + 14)		
<i>S. neornaticipes</i> "2"	X_0, Y_1 (3L-17), Y_2 (3L-17 + 23)		
<i>Simulium pictipes</i> group			
<i>S. pictipes</i> "B"	X_0, Y_1 (3L-3)		Bedo (1975)
<i>S. longistylatum</i>	X_0, Y_0, Y_1 (1S-1)		
<i>Simulium vittatum</i>	X_0, Y_0, Y_1 (3L-1)		
<i>Simulium ornatum</i> group			
<i>S. ornatum</i> "C"	X_1 (2S-1), Y_0, Y_1 (2S-1)—ancient polymorphism		Pasternak (1964) Post (1979) and Rothfels (1979)
<i>S. ornatum</i> "B"	X_1 (2S-3), Y_0, Y_1 (2S-3)—ancient polymorphism		
<i>S. nitidifrons</i>	X_0, Y_0, Y_1 (un-named)		
<i>Simulium tuberosum</i> group		Y_0 and Y_1 allopatric	
<i>S. tuberosum</i> "CKL"	X_1 (2S-C, K, L), Y_0 (2S-A, ancient polymorphism)		Landau (1962)
<i>S. tuberosum</i> "AB"	X_1 (2S-A ancient polymorphism + B), Y_0 (St)		
<i>S. tuberosum</i> "FG"	X_1 (2S-F, G), Y_0		
<i>S. tuberosum</i> "CDE"	X_0 (2S-A ancient polymorphism), Y_1 (2S-C, D, E) X_0, Y_1 (un-named double rearrangement)		
<i>Simulium decorum</i>			Rothfels (1979) Rothfels, Feraday and Kaneps (1978)
<i>Simulium venustum</i> / <i>verecundum</i> group			
<i>S. verecundum</i> "ACD"	X_1 (2L-5 . 6 + 4), X_2 (2L-5 . 6 + 4 + 8 + 9), X_3 (2L-5 . 6 + 4 + 7), X_4 (2L-5 . 6 + 4 + 7 + 9 + 10), X_5, X_6, X_m, Y_1 (2L-1 . 2), Y_2 (2L-1 . 2 + 14), Y_3, Y_n	2L-1 . 2 and 4 are ancient polymorphisms, X_{5-6} are derived independently from other X chromosomes by single inversions (2L-11 and 13). Similarly Y_3 from an unstated other Y by single inversion (2L-15). The authors do not name but state that "several" other Y and many other X sequences occur (Y_n and X_n)	

TABLE 1—continued
Sex linked inversions in blackfly species

Species	Sex linked inversions	Notes	Authors
<i>S. verecundum</i> "A/C"	X_0 (2S-A), Y_0 (St)—ancient polymorphism	The "A" sequence is assumed to have become sex-linked independently in the two species	Post (1979)
<i>S. venustum</i> "A/C"	X_0 (2S-A), Y_0 (St)—ancient polymorphism		
<i>S. venustum</i> "EFG/C"	X_0 (2S-E, F, G), Y_0 (St)—ancient polymorphism		
(= <i>truncatum</i>)			
<i>S. venustum</i> "CC1"	X_0 (3L-5 ancient polymorphism), Y_1 (3L-6)		
<i>S. venustum</i> "CC2"	X_0 (2L-1), Y_0 (St)—ancient polymorphism		
<i>Simulium erythrocephalum</i>	X_0 , Y_0 , Y_1 (1L-Q, R, S, T), Y_2 (1L-Q, R, S, T, + 1S-P), Y_3 (1L-Q, R, S, T, + 1S-P+H) Also: 1S-B & 1, G, have higher frequency on X, and 1L-A has higher frequency on Y		
<i>Simulium aureum</i> complex		The complex is male chiasmate	Dunbar (1958) and Dunbar (1959)
<i>S. aureum</i> "C"	X_0 , Y_0 , Y_1 (1C-indeterminate rearrangement)		
<i>S. aureum</i> "D"	X_0 , Y_0 , Y_1 (1S-9), Y_2 (1S-9+16)		
<i>S. aureum</i> "C"	X_0 , X_1 (1S-3 ancient polymorphism), Y_1 (1S-13, 15), Y_2 (1S-13, 15+14)		
<i>Simulium congaerenanum</i> group		X_0 and X_1 allopatric	Dunbar (1967)
<i>S. congaerenanum</i>	X_0 , Y_1 (1S-3)		
<i>S. innocens</i>	X_0 , Y_1 (1S-3+4)	Nucleolar organiser inactive on Y Y is derived from <i>S. congaerenanum</i> Y, and also anucleolate	
<i>S. excisum</i>	X_0 , Y_1 (3S-21, 23, 24)		
<i>S. rivuli</i>	X_0 , Y_1 (3L-27)		
<i>Cnephia</i> (<i>Cnephia</i>) <i>pecuarum</i>	X_0 , Y_1 (1S-5)		
<i>Cnephia</i> (<i>Stegopterna</i>) species		Sub-genus <i>Cnephia</i> is male chiasmate	Rothfels (1979) Madahar (1969), Basrur and Rothfels (1959)
<i>C. mutata</i> (diploid form)	X_0 , Y_1 (indeterminate pericentric rearrangement—chromosome 1)		
<i>C. "O"</i>	X_0 , Y_1 (un-named complex rearrangement—chromosome 1)		
<i>C. "W"</i>	X_0 , Y_1 (un-named complex rearrangement—chromosome 2)		
<i>Prosimulium hirtipes</i> group		Y_0 and Y_1 allopatric	Basrur (1959), Rothfels (1979) and Post (1979)
<i>P. hirtipes</i>	X_0 , X_1 , (1S-10), Y_0		
<i>P. arvense</i> / <i>viginquartem</i>	X_1 (1S-14, 15), X_2 (1S-14, 15+17), Y_0 , Y_1 (1S-16)		

<i>P. latimucro</i> (= <i>inflatum</i>)	X_0, Y_0, Y_1 (1C-1 . 1L-6)	Y_0 and Y_1 allopatric, also nucleolar organiser inactive on Y	Basrur (1959), Rothfels and Freeman (1977) and Rothfels (1979)
<i>Prosimulium mixtum</i> group <i>P. fontanum</i> <i>P. mixtum</i>	X_0, Y_1 (1S-2 . 3 . 4 . 5 . 6 . 7) $X_0, X_1, X_2, X_3, X_4, Y_1$ (3C-1), Y_2 (3C-1 + 3S-2), Y_3 (3C-1 + 3S-3), Y_4 (3C-1 + 3S-4), Y_n	X_1-X_4 are 4 pericentric rearrangements independently based on X_0 . 4 further Y -linked inversions occur, but it is not stated in which combinations they occur (Y_n)	
<i>P. fuscum</i>	$X_0, [X_1 (3S-X)], X_2 (3S-X + \text{un-named inversion}), [Y_1 (2S-Y)], Y_2$ (a second inversion, based on Y_1), Y_3 (a third inversion, based on Y_1)	There is presumed to be some recombination between X_1 and Y_1 because the alternate sequences are not quite fixed on the different sex chromosomes, although X_2, Y_2 and Y_3 are. The authors also mention that there are rare additional X and Y inversions	
<i>P. saltus</i>	X_0, Y_1 (3L-inversion), Y_2 (a second inversion based on Y_1)		
<i>P. approximatum</i> <i>P. mysticum</i> <i>P. rhizophorum</i>	X_0, Y_1 (3L-inversion) X_1 (3L-inversion), Y_1 (3S-inversion) X_0, X_1 (3S-inversion), Y_0		
<i>Prosimulium magnum</i> group <i>P. impostor</i> <i>P. dicum</i>	X_0, X_1 (1S-2), Y_0, Y_1 (1C-1) X_0, Y_0, Y_1 (1S-indeterminate rearrangement), Y_2 (1S-another indeterminate rearrangement), Y_3 (1S-inversion, based on Y_2) X_0, Y_1 (1S-1), Y_2 (1S-1 + 13), Y_3 (1S-1 + 3), Y_4 (1S-1 + 4) X_1 (3C-X), Y_1 (3C-Y), Y_2 (3C-Y + 3L-1), Y_3 (3C-Y + 3L-2 . 3), Y_4 (3C-Y + 3L-2 . 3 + 4 . 5)	It is not clear if Y_2 is based on Y_1	Ottonen (1966), Ottonen and Nambiar (1969) and Rothfels (1979)
<i>P. multidentatum</i>			
<i>P. magnum</i> "form 3" (= <i>albionense</i> ?)			
<i>Simulium damnosum</i> complex (W. Africa) <i>S. squamosum</i> <i>S. yahense</i> <i>S. soubrense</i> / <i>S. sanctipauli</i> <i>S. damnosum</i> s.s. <i>S. sirbanum</i>	X_0, Y_0, Y_1 (1C-indeterminate rearrangement) 2L-18 at higher frequency on X than Y 2S-6b at higher frequency on Y than X 2L-8 and 3-8 at higher frequency on Y than X 1S-3 ancient polymorphism	Samples from Dahomey Gap only The 1S-3/st polymorphism is linked to sex in a complex and as yet undetermined manner	Vajime and Dunbar (1975), Garms, Meredith and Cheke (in press), and Post (unpublished)

Most blackflies are male chiasmate, but a few are not and these are noted where appropriate. Such species are not included in table 2, which compares the total numbers of inversions designated to each category of the classification.

3. DISCUSSION

Modern theory seeks to explain the selection of any new inversions as a consequence of their effect on recombination. Thus an inversion which chances to link two epistatic genes will increase in frequency if selection is already acting to produce linkage disequilibrium at these loci (see amongst others, Charlesworth and Charlesworth, 1973; Hartl, 1977). Sex-linkage is probably just a special case of this, where one of the genes concerned is the primary sex determining locus (primary sex determination in blackflies is probably controlled by a single locus, or at most by a very short length of chromosome, because most species have indistinguishable sex chromosomes and males are usually chiasmate). This being so how might we explain the different classes of sex-linked inversions observed in blackflies?

White (1978) has suggested that if a locus shows heterozygote advantage only in the male then there will be selection for sex linkage such that all males will be heterozygous. Charlesworth and Charlesworth (1980) have investigated this model numerically and shown that structural rearrangement is more likely to involve the *Y* chromosome, because *Y* chromosome rearrangements whilst polymorphic are more efficient at preventing recombination between the primary sex locus and the other selected locus. It is not clear how much this prediction will be affected by the greater likelihood of a suitable new inversion occurring first on the *X* chromosome, but the data from blackflies do appear to support the model, in that they show a clear excess of *Y*-linkage over *X*-linkage. We see this excess in both fixed and polymorphic sex-linked inversions, although the White/Charlesworths hypothesis predicts only fixation of inversions. It may be possible to explain these polymorphic inversions by introducing frequency dependence into the model, such that the heterozygote advantage is conferred on only a given proportion of the male population; the others experiencing the same selective pressures as the females.

The large class of inversions which I call "ancient polymorphisms" is of interest in that they presumably were originally selected for reasons not concerned with sex. However, it seems that subsequently their genetic effects have become modified, possibly by mutation of included genes, such that sex linkage became advantageous, and this linkage has been achieved, not by further structural rearrangement but possibly by genic modification of recombination. Changes in the genetics of autosomal inversions have already been documented in *Drosophila* by Dobzhansky and Pavlovsky (1958).

There are nine inversions recorded which occur on both *X* and *Y*, but at different frequencies. These may include genes which interact epistatically with the primary sex locus, which is itself outside the inversion and recombines with it thus maintaining a linkage disequilibrium. Post (1979 and 1980) has analysed such a polymorphism in *S. erythrocephalum* and found that heterozygosity was associated with faster larval development.

TABLE 2
Numbers and classification of sex-linked inversions in blackflies

Class of sex linkage	1a Fixed to Y	1b Fixed to X	2a Polymorphic Y	2b Polymorphic X	3 Ancient polymorphism	4 Different frequencies
Number of inversions	20	8	30*	15	14	9

* It is not always clear from the author's description whether an inversion is polymorphic or polytypic. This value includes at least three cases of polytypism.

This is probably an advantage to males, which then have potentially more mates, but faster females are smaller and probably lay fewer eggs. If no other factors were effective in *S. erythrocephalum* we would expect selection for the further suppression of recombination and the eventual fixation of the alternate sequences to X and Y.

In the absence of any specific model it is difficult to assess the hypothesis (Bedo, 1977; White, 1978) that sex chromosome rearrangement may be important in blackfly speciation. However, the observations that the karyotypes of closely related species often differ only in the sex chromosomes (Rothfels, 1979) and only very rarely share the same differentiated sex chromosome pair (table 1) are suggestive. The process described above for the evolution of genes which interact epistatically with sex could result in changes in the sexual system such that either pre- or post-zygotic isolation developed between populations according to any of several speciation models discussed by White (1978).

So far we have only considered sex-linked inversions and selection for decreased recombination between two epistatic genes, one of which is the primary sex determining locus. The existence of nine inversions occurring in apparently stable linkage disequilibrium with the sex chromosomes suggests that this model is realistic, but does not exclude the possibility of others. Indeed the discovery of sex-linked inversions in the *S. aureum* complex, which is male achiasmate, suggests that other processes must be important.

Inversions which happen to include the primary sex locus but are selected for other reasons will be unable to cross over onto the other sex chromosome and will hence be restricted in how they spread through the population. Such associations may very well be disadvantageous and there may be selection to break them, possibly by moving the site of primary sex determination to another position in the karyotype. Movement of the primary sex locus is inferred to be commonplace from the frequent differences in sex chromosome pair observed between closely related species of blackflies (see table 1).

Neutral inversions which happen to include the primary sex locus are more likely to reach high (detectable) frequencies than neutral autosomal inversions, because they will be restricted to one or other of the sex chromosomes and therefore exist in an effectively smaller population. This is particularly true of the Y-chromosome. Drift of this sort is likely to be more important if blackfly populations occasionally pass through bottlenecks, as is proposed under several general models of speciation (White, 1978).

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