

know, one thing which seems clear is the need to act now, as climate change has been shown to have a significant impact on arid regions including centers of endemism of the Euryphyminae (Foden et al. 2007).

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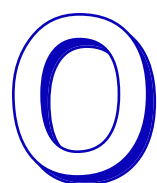
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## Evolutionary recycling of sex chromosomes in Neotropical Melanoplinae

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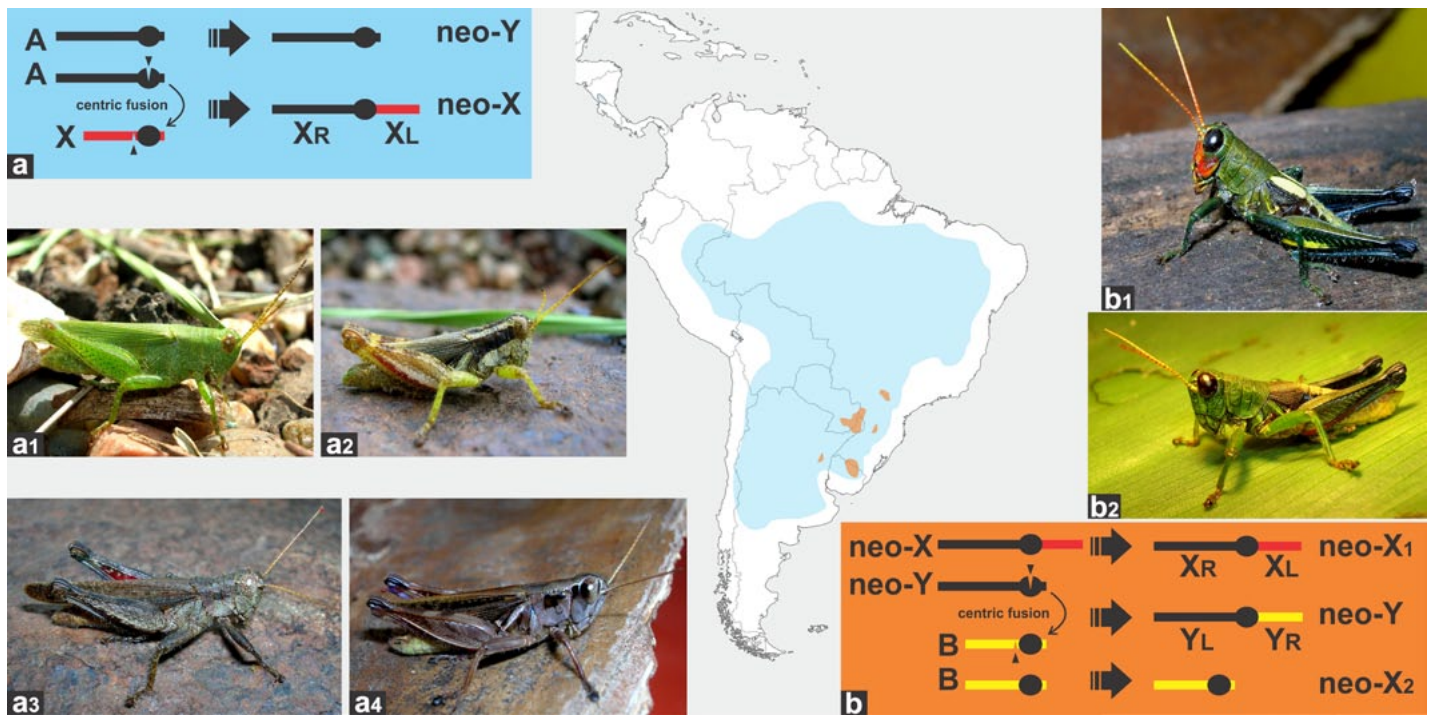
Orthoptera are a fascinating group of insects in multiple ways. My own research focuses on the evolutionary relationships of South American acridoid grasshoppers with emphasis in the Melanoplinae. In this respect, evidences from different research areas, such as taxonomy, morphometrics, molecular genetics, and cytogenetics are of relevance for the understanding of the evolutionary history of these species, which is my main objective. In this report I will summarize some cytogenetic characteristics of melanoplinae and highlight their biological relevance.

Pioneering cytogenetic research has shown that acridid grasshoppers display an apparent karyotypic stability, with the vast majority of species exhibiting a standard (and presumably ancestral) acro-telocentric karyotype ( $2n=23\text{♂}/24\text{♀}$ ;  $FN=23/24$ ) including an XO/XX chromosomal sex determination mechanism. Nevertheless, evo-

lutionary structural modifications of the standard karyotype due to different types of chromosomal mutations have occurred along their history. There are instances of reduction in chromosome number with no morphological variation of chromosomes, presumably due to tandem fusions, an unusual kind of chromosome mutation (i.e., *Dichroplus pratensis* with all-telocentric  $2n=19/20$  or some species of *Trimerotropis*). Reduction of the number of chromosomes without change in the fundamental number (FN) through centric fusion has also occurred and is manifested as fixed interspecific differences (either autosomal or involving sex chromosomes, see below) or, more frequently, polymorphisms. Intraspecific variation due to polymorphic centric fusions (i.e. *Dichroplus pratensis* Bidau & Martí 2002, and *Dichroplus fuscus* Taffarel et al. 2014) or pericentric inversions (i.e. *Trimerotropis* spp Guzmán & Confalonieri, 2010) are important sources of chromosomal variation in

natural populations. Besides, some species have proved to be excellent experimental models to study how chromosomal rearrangements occur and affect critical features of the genetic system, e.g recombination (Castillo et al 2010a, b; Castillo et al. 2014).

One of the most important components of chromosome variation in Acrididae is constituted by neo-sex chromosome systems, which arose independently from standard XO/XX systems within several lineages. In Acrididae, species karyotypes show a trend toward the fixation of centric fusions; the cytogenetic evidence in an important number of melanoplinae supports this premise, showing species with derived karyotypes and neo-sex chromosome mechanisms (Bidau & Martí 2001; Castillo et al. 2010 a,b; Bidau et al. 2011). In general terms, when there is centromeric breakage of the X chromosome and an autosome, and subsequent fusion occurs, a neo-sex chromosome arises (Castillo et al.



**Figure 1.** Schematic view of neo-sex chromosome formation produced by centric fusion a) between an Autosome (A) and the X chromosome. Male individuals of a1 *Atrachelacris unicolor*, a2 *Ronderosia malloi*, a3 *Ronderosia forcipata*, a4 *Dichroplus obscurus*; b) between the neo-Y and another autosome, giving rise to a neo-X1X2Y complex sex-chromosome mechanism. Male individuals of b1 *Dichromatos schrottkyi*, b2 *Dichromatos lilloanus*. The South America map shows the potential distribution of melanopline species with neo-XY (in blue) and with complex sex chromosome mechanisms (in orange).

2010 b). This neo-X chromosome is formed by the XL arm (the ancestral X chromosome) and the XR arm (the ex-autosome), and the homologue of the fused autosome is now called the neo-Y (Fig. 1a). While the mechanism of the physical chromosome rearrangement per se is not difficult to understand, the evolutionary implications of this phenomenon require a more detailed study. In recent publications we have had the opportunity to accurately describe and discuss several interesting cases (Castillo et al. 2010 a,b; Bidau et al. 2011; Castillo et al. 2014).

Acridid neo-sex chromosomes allow the addressing of one of the most exciting issues in evolutionary biology, that of the emergence and divergence of sex chromosomes. In general, classic theory of sex chromosome evolution proposes as the starting point, the appearance of a sex-determining gene in a pair of ordinary autosomes. Later on, sex determining and/or sex-related genes begin to be inherited together due to abolition of recombination near

the new sex region, which drives the XY divergence process (Charlesworth et al. 2005, Kejnovsky et al. 2009). While this is so in most of the studied models, in Orthoptera the events could follow a different path (Castillo et al. 2010 a,b; Bidau et al. 2011, Castillo et al. 2014). Neotropical Melanopline species are ideal models for the study of sex chromosome evolution because many cases of neo-sex chromosomes at various evolutionary stages are known (Hewitt 1979, Castillo et al. 2010 a,b). The amazing diversity of neo-XY chromosome systems in this group is due mainly to X-A centric fusions that occurred repeatedly in the evolutionary history of several lineages (Bidau & Martí 2001, Mesa et al. 2001; Castillo et al. 2010 a,b; Castillo et al. 2014). Probably however, the most important feature of South American Melanopline neo-sex chromosomes is that several evolutionary instances defined by particular cytogenetic properties have been identified (Castillo et al. 2010 b). Only a few species show a small

degree of differentiation, indicative of a recent origin, where the neo-Y still conserves high homology with the XR arm of the neo-X. Recently arisen simple neo-sex chromosome systems will be initially represented by a sex pair, which still conserves almost the whole of the homology, synaptic ability, and possibility of free recombination along the fused autosome (XR) and its homologue, the neo-Y (e.g. *Oedaleonotus enigma* Hewitt & Schroeter 1968). On the other end of the cytogenetic spectrum, there is almost complete loss of homology between Y and XR, evidenced by an extremely reduced synaptic region, exclusive distal meiotic association, accumulation of repetitive heterochromatic sequences in the Y, and eventually the possibility to fix complex structural rearrangements (examples of this “terminal” stage are *Ronderosia bergii* [Sáez 1963, Cardoso & Dutra 1979] and *Dichroplus vittatus* [Bidau & Martí 2001]).

Between these two cases, a continuous spectrum of neo-sex chromosome

conditions revealing different evolutionary strata are found (White 1973, Hewitt 1979, Castillo et al. 2010a, b). Some examples can be mentioned as follow: *Dichroplus obscurus*, *Atrachelacris unicolor*, *Ronderosia forcipatus*, *R. malloi*, *Eurotettix minor*, *Zoniopoda iheringi*: the sex pair of these species is formed by a metacentric neo-X, product of the centric fusion of the ancestral X and an autosome while the homologue of the translocated autosome becomes the telocentric neo-Y. Despite the same mechanism proposed for the neo-sex chromosome system of these species, the neo-sex pair involved different pairs of autosomes, at least at the generic level. An example of a highly evolved neo-XY system was studied in *Dichroplus silveiraguidoi*. This species is unique in being  $2n=8$  (see above) and the only one known in which the neo-X is telocentric, the neo-Y being very small and acrocentric (Sáez 1957). Due to this extraordinarily re-arranged karyotype it is very difficult to infer the evolutionary history of the sex chromosomes (Cardoso et al. 1974, Cardoso & Dutra 1979). *Dichroplus vittatus* and its close relative *D. maculipennis* have complex neo-XY systems, which are not interpretable in classic terms (Bidau & Martí 2001; Mesa et al. 2001; Castillo, Martí & Bidau, unpub.) An unusual origin of neo-sex chromosomes was recently proposed for *Boliviacris noroestensis* (Castillo et al. 2014).

In a further leap of complexity, a neo-XY system may undergo a conversion into an X1X2Y system through a Y-autosome fusion if a second pair of telo/acrocentric autosomes is available to become incorporated into the sex chromosome mechanism (Fig. 1b) (White 1973, Hewitt 1979, Castillo et al. 2010 a,b). Although much less frequent than typical neo-XY chromosomes the majority of these complex systems correspond to Neotropical species of restricted geographic distribution (Castillo et

al. 2010 b). All neo-X1X2Y species belong to genera in which neo-XY mechanisms are frequent (i.e. *Ronderosia*, *Scotussa*), or all known species share this type of sex chromosomes (*Dichromatos*). Since X1X2Y mechanisms derive from previous XY systems through centric fusion of the neo-Y with a second autosome (the unfused element becoming X2), it is probable that all known cases have originated from advanced XY systems. This is reasonable, considering that the probability of rapid occurrence of two sequential fusions is very low (Castillo et al. 2010 b).

The cytogenetic evidence suggests that the replacement of X0-XX for XY-XX was favored many times in Neotropical Melanoplinae. The high frequency of neo-sex chromosome determination systems, and their independent origins point to a higher incidence of chromosome rearrangement within this group. If this is so, are centric fusions (or other rearrangements involved in neo-sex chromosome formation) random chromosome restructures due to events of non-homologous recombination? Is selection involved in neo-sex chromosome maintenance in natural populations or is it genetic drift? (Veltsos et al. 2008)

Differently from other insect groups, little is known about the intimate mechanism of sex-determination in Orthoptera and most derives from extrapolation of theoretical and empirical data of other biological systems (White 1973; Pannell & Pujol 2009; Kaiser & Bachtrog 2010). Thus, the main question in this respect is: are neo-sex chromosomes in Orthoptera subject to the classical path of sex chromosome evolution? All the cytogenetic evidence currently available points to a different path, for whose elucidation new molecular evidence is necessary, especially relating to neo-Y chromosome degeneration and the mapping of sex-determining genes in the new sex chromosomes in this particular group. Also, despite

descriptive studies of neo-XY chromosomes in Melanoplinae nothing is known about their meaning in evolutionary terms and their role in sex determination remains a mystery in Orthoptera. The description and understanding of neo-sex chromosomes' structure, meiotic behavior, and their origin in species of Orthoptera is a little-explored field and actually deserves more attention than it has received (Bidau et al. 2011).

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## My Resilient and Surprising Colony of Orthoptera

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The summer during which I was supported by the Orthopterists’ Society things did not go as planned. My project was stalled and would later have to be completely overhauled due to the mechanical engineer’s difficulties in building the needed equipment. The females in the *Gryllus firmus* stock I had been working with did not seem to find the same males attractive as those in previous generations. Worst of all, the stock cages became overrun with Argentine ants looking for water and food in the near-desert southern California climate. Every day would bring new casualties in my stock cages with several individuals being swarmed by ants in each cage. Since most of the usual tricks of fumigation or insecticide spray would also harm my model species I had to

rely on improvised measures. Some of the grant money from the Orthopterists’ Society was used for water trays under the cages, bleach solution used as insecticide spray on the trails of ants outside the cages, caulk for plugging up ant entry ways, and a multitude of other devices in the war against the unwanted invaders.

Some of the crickets that were saved were used as the parent generation for a population to be used in a female preference study, also supported by the Orthopterists’ Society grant. Specifically I was looking into



the repeatability of female choice for the song of male crickets in *G. firmus*. *G. firmus* is a dimorphic species with short-winged and long-winged individuals. What was known from previous experiments in my lab is that females prefer the short-winged morph. The results from my repeatability