

RESEARCH NOTE

Male-biased recombination in odonates: insights from a linkage map of the damselfly *Ischnura elegans*

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Introduction

Odonata (dragonflies and damselflies) represent the most ancient insect branch and have received much attention in the evolutionary and ecological research. However, few genomic resources are available and no genetic maps or genome assemblies have been developed. We present recombination rates and a first-generation linkage map of the blue-tailed damselfly *Ischnura elegans*. Using a pedigree, 19 microsatellite markers were genotyped, resulting in four linkage groups with a total length of 212 cM. Interestingly, hemigametic males (332 cM) had a moderately larger linkage map than homogametic females, (179 cM), suggesting that recombination in damselflies is heterochiasmatic. This contrasts with the situation in some more modern insect orders such as some Diptera and Lepidoptera, where the heterogametic sex is achiasmatic, or in some Orthoptera, where females have higher recombination rates. Apart from providing insights into insect recombination patterns, this linkage map forms a valuable genetic resource for future research in Odonata.

Almost a century ago, the work on recombination and genetic linkage in *Drosophila melanogaster* by Morgan (1914) and Sturtevant (1913) laid the foundation for classic genetics and linkage mapping. Since then, linkage maps have been produced for a wide variety of animals and plants. Both large differences in recombination rates such as ‘achiasmatic recombination’, where one sex does not recombine, or ‘heterochiasmatic recombination’, where the sexes recombine to different degrees, have been reported (reviewed in Burt *et al.* 1991; Lenormand and Dutheil 2005). Evaluating how different recombination characteristics are distributed

among species is valuable, since it yields insights into the evolution of recombination patterns (Lenormand and Dutheil 2005). However, linkage maps are still lacking for many model organisms in ecology and evolution, including all species within the ancient insect order Odonata (dragonflies and damselflies).

Odonata and Ephemeroptera (mayflies) are the basal lineages of winged insects (Pterygota). In contrast to the recent successes in resolving the evolutionary relationships of Holometabola and the origin of Hexapoda, the relationships of the earliest-diverging lineages of winged insects remains unsolved (Trautwein *et al.* 2012). Odonates have received much attention as a model organism in ecology, evolution and conservation biology (Córdoba-Aguilar 2009). In particular, odonates are emerging model systems for biotic effects of climate change and in sexual selection research (Wellenreuther *et al.* 2012).

Here, we present the first data on recombination rates and sex-biased recombination in odonates and a first-generation linkage map of *I. elegans* (blue-tailed damselfly). *I. elegans* and its sister species *I. graellsii* have been intensively studied with respect to sexual conflict, frequency dependent selection, molecular population differentiation and hybridization (Svensson *et al.* 2005; Sánchez-Guillén *et al.* 2011, 2012; Wellenreuther *et al.* 2011). Estimating recombination rates will be useful to the wider research community, since it will open up new research opportunities in this insect group, such as examining the degree of linkage disequilibrium in different populations and the genetic basis of quantitative traits. Recombination rates and mapping data of odonates will be important to understand the evolution of achiasmy and heterochiasmy within insects in general. We therefore compared sex-biased recombination in *I. elegans* obtained in this study with data from other insects available in the literature.

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Materials and methods

Ischnura elegans belongs to the largest damselfly family Coenagrionidae, which includes 95 genera and 1082 species worldwide (Paulson 2009). Its karyotype consists of 13 bivalent chromosomes and one large univalent X-chromosome ($2n = 28$) of roughly equal size. *I. elegans* females are homogametic (XX) whereas males are hemigametic (X0) (Frydrychová et al. 2004).

A laboratory-bred pedigree of *I. elegans* and *I. graellsii* was used in this study for the construction of the linkage map. The pedigree was founded with 13 individuals consisting of seven females and six males from Spain of which all males were *I. elegans* males but one of the females was an *I. graellsii* and one an F₁ hybrid between *I. graellsii* and

I. elegans. Two hundred and twelve individuals that span over three generations were finally obtained. The individuals were stored in 70% ethanol for subsequent DNA extractions. DNA was phenol/chloroform – isoamyl alcohol extracted from 192 individuals.

Microsatellite polymorphism was tested among four unrelated individuals by screening a set of published (Wellenreuther et al. 2010) and unpublished (see table 1 in electronic supplementary material at <http://www.ias.ac.in/jgenet>) microsatellite loci. Primer sequences and PCR amplification conditions of the microsatellites are given in table 1 in electronic supplementary material. PCR products were separated and visualized using an ABI3730 capillary sequencer and analysed in GeneMapper 4.0 (Applied

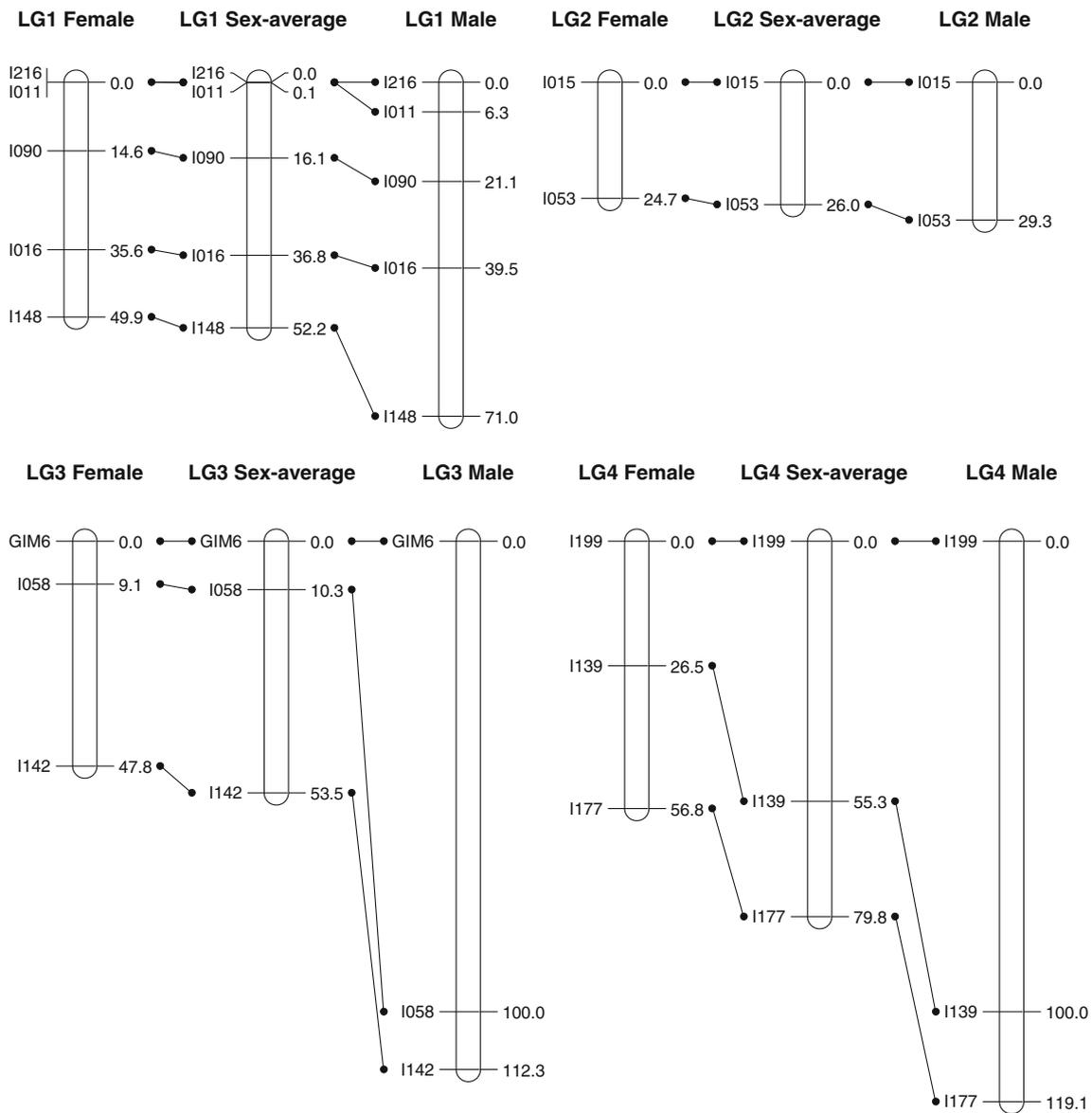


Figure 1. Sex-specific and sex-average linkage maps of *Ischnura elegans*. Female linkage groups are to the left and male maps to the right in each linkage group. Shown is the most parsimonious map. Genetic distances are given in cM.

Odonate linkage map

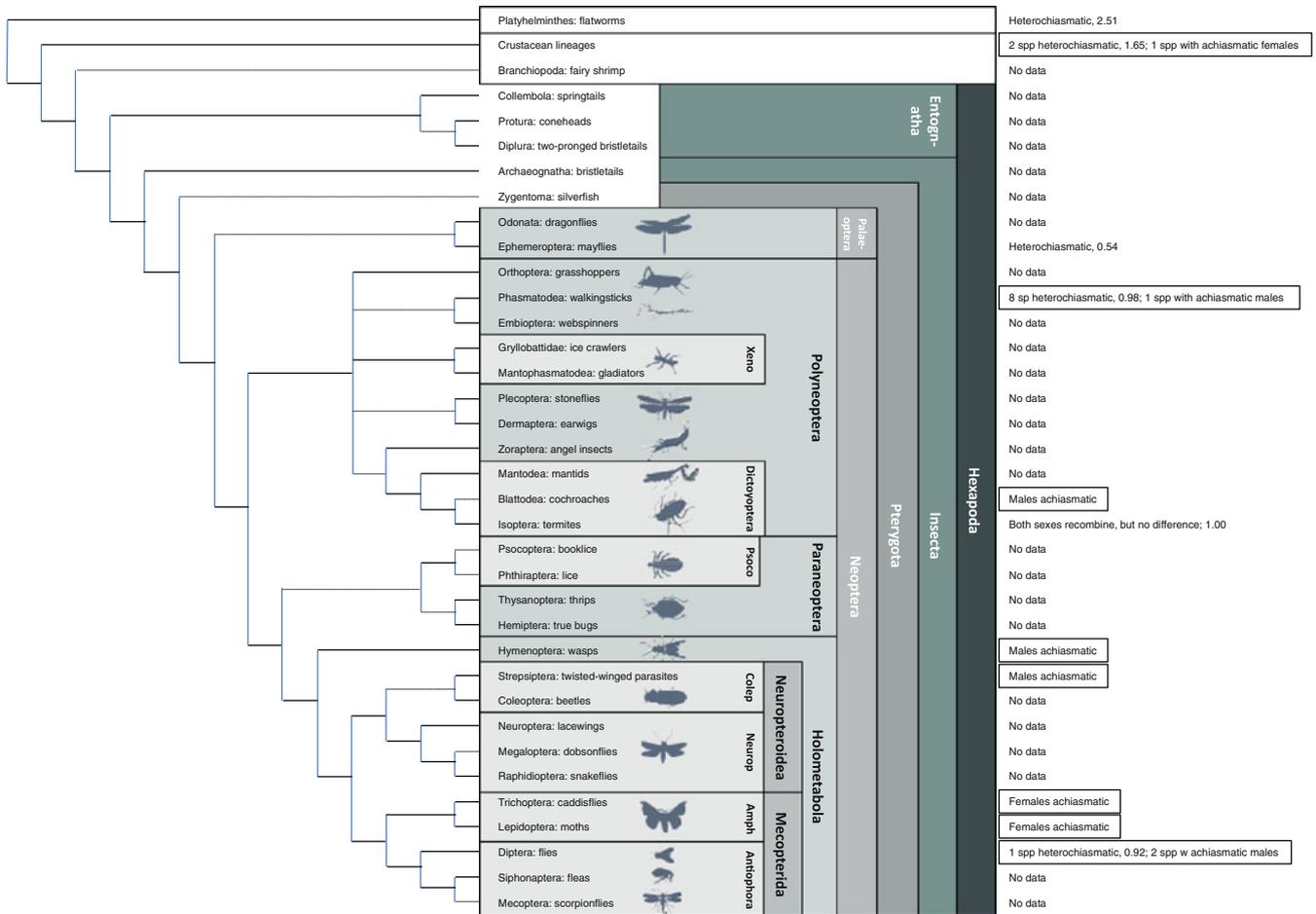


Figure 2. Sex specific recombination rates in Insects and Platyhelminthes. Phylogeny modified from Trautwein *et al.* (2012). Species details are given in table 2 in electronic supplementary material. When recombination ratios of more than one species were available for an order, then the average is given. Orders with achiasmatic species are shown as black rectangles. In cases where both heterochiasmatic and achiasmatic species occur within the same order, the number of species following either pattern is stated.

Biosystems, Foster City, USA). Null-alleles were present at a few loci in some families and the segregation of these alleles was accounted for in the analyses.

Genetic linkage between markers was analysed in CriMap v. 2.4 (Lander and Green 1987). As in previous mapping studies (Hansson *et al.* 2010), we used two-point recombination analyses to assign markers to linkage groups at a threshold of a logarithmic odd score, LOD, >3.0 (three markers, I199, I139 and I177, were assigned using LOD > 2.0). We determined the most parsimonious ordering (order with highest likelihood support) of markers within each linkage group with the options BUILD, FLIPSN and FIXED. Heterochiasmy was determined with sex-specific analyses. Map distances are given in Kosambi cM (Kosambi 1944).

Results

The two-point analyses detected that 13 of the 19 markers were linked to at least one other in our *I. elegans* mapping data set. These markers built up four linkage groups with two

to five markers (figure 1). No markers were linked to more than one linkage group, and thus no conflicting assignments occurred.

The parsimonious sex-average autosomal linkage map spanned 211.5 cM. A moderate degree of heterochiasmy was found, with a female map of 179.2 cM and a male map of 331.7 cM (paired *t*-test on log₁₀ map distances at the four linkage groups: *t* = 3.295, *df* = 3, *P* = 0.046). The female-to-male map ratio was 0.54, i.e. -0.27 on a log₁₀-scale.

To compare the pattern of recombination in insects, we compiled data of sex-specific recombination, either from linkage mapping studies or recombination nodule counts, for 30 insect species (including *I. elegans*) (figure 2; details and references are given in table 2 in electronic supplementary material). Comparison of recombination rates within the class of Insecta showed that most species have higher recombination in females, contrasting the pattern found for *I. elegans*, and that achiasmatic recombination is widespread across multiple orders.

Discussion

Striking sex differences in recombination rates across the animal and plant kingdom have been known for decades (Burt *et al.* 1991; Lenormand 2003; Lenormand and Dutheil 2005), but the record is incomplete and prevents general conclusions. This is particularly true for the class Insecta. In fact, *I. elegans* recombination data presented here are the first for the ancient order Odonata.

Based on figure 2, we conclude that achiasmatic recombination is widespread in Insecta, and has probably evolved independently in several orders e.g. in Diptera (Morgan 1914) and Lepidoptera (Tanaka 1914). Heterochiasmy, on the other hand, is rarer, which could partly be a publication bias, as researchers might report only the more extreme cases of sex-biased recombination (such as achiasmy). Heterochiasmatic data would be expected to be available from model species such as *Apismellifera* and *Tribolium castaneum*, which have been the subject of linkage mapping and genome assembly projects (<http://www.ncbi.nlm.nih.gov>), but computations were done on sex-averaged maps and sex differences were not reported.

Early studies in plants and animals suggested a correlation between achiasmy and heterogamety. In Diptera, males are the heterogametic and nonrecombining sex, while in Lepidoptera, females are heterogametic and nonrecombining. This was formalized into the so-called Haldane–Huxley rule (Haldane 1922; Huxley 1928). This rule was thought to be a side effect of the suppression of recombination between the sex chromosomes, resulting in recombination suppression also on autosomes (reviewed in Burt *et al.* 1991; Lenormand and Dutheil 2005). However, several heterochiasmatic species do not seem to obey the Haldane–Huxley rule (Hansson *et al.* 2005; Lenormand and Dutheil 2005). Within insects, the general pattern that is emerging is that the degree of heterochiasmy varies substantially between species; ranging from pronounced female-bias (with a female-to-male ratio of 1.21 in the Orthopteran grasshopper *Stethophymagrossum*) to a moderate male-bias in recombination *Anopheles gambiae* (0.92) and in *I. elegans* in this study (0.54) (figure 2). As females are the homogametic sex in *Ischnura*, but nevertheless have a lower recombination rate than males, this further suggests that the Haldane–Huxley rule does not hold for species that are heterochiasmatic.

Consequently, the lack of a clear association between heterogamety and heterochiasmy implies that other hypotheses have to be invoked to explain the existence of sex-dimorphic map distances. Several alternative explanations to heterochiasmy have been proposed. For instance, heterochiasmy might arise from differences in selection. It has been suggested that sex-specific epistatic gene interactions during the haploid phase might lead to recombination suppression in one sex in order to minimize the recombination load (Lenormand and Dutheil 2005). Another hypothesis implicates sexual selection, with reduced recombination in the sex with highest variance in reproductive success (Trivers

1988). Data on sex-specific recombination rates, life-history patterns and demography are clearly needed before we can understand and explain the large observed variation in the degree of heterochiasmy in insects.

In conclusion, the emerging view from this and previous insect mapping studies is that a great diversity of recombination frequencies exists within the class Insecta and that both achiasmatic and heterochiasmatic patterns vary between groups. The findings in this study should stimulate more detailed research on sex-differences recombination frequencies in taxa with different life histories or ecological settings to better understand the evolutionary causes for these large-scale patterns in insects and other taxa.

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