Chromosomal evolution of *Delena cancerides*

Hayley Sharp

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Although the bulk of this thesis has been researched and written independently, several other people have made substantial contributions:

Chapters 1 & 2. Dr David Rowell (supervisor) co-authored Chapter 1, contributing mostly background information regarding chromosomal speciation.

Chapter 3. Dr Scott Keogh (advisor) ran the Bayesian phylogenetic analysis.

Chapter 4. Dr Terry Neeman (ANU Statistical Consulting Unit) advised on the analysis methods for the fertility data, and thought of the solution shown in Figure 7 B.

Chapter 5. Dr Mike Double (advisor) wrote the Excel macro referred to as the fusion model in (Appendix 1). Dr Neeman developed the mathematical framework with which to independently verify the fusion model, and the R program for calculating the probability of shared fusions (Appendix 2). The author's contribution to these aspects of the thesis was advising on the design of the tools, and interpreting the results.

Chapter 6. Dr Neil Murray contributed extensively to the Speculation section of this chapter, providing many helpful discussions and suggesting that position effects and dosage compensation could be relevant.

Aaron Henderson provided invaluable IT assistance, and constructed several of the figures (Chapter 2 figures 2 & 4; Chapter 3 Figure 2; Chapter 4 Figures 3, 6 B & 7 B; Chapter 5 Figure 1).

With the exception of the above contributions, this thesis is entirely my own work.
Signed:

This thesis is dedicated to the memory of my grandfather Ernest Cormick who taught me to love nature and respect science. He showed me how to use a microscope shortly before his death on the 5th of February, 1987.

Many spiders were harmed in the production of this thesis. May their lives not have been taken in vain.

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Academic

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Abstract

Chromosomal evolution has long been linked with the process of organismal speciation, and many different theories have been suggested over the years to explain why this would be so. These theories can be loosely grouped into two eras. Classical chromosomal speciation models focused on negative heterosis of chromosomal rearrangements causing malsegregation and germ cell death in hybrids. More recent models examine the effects of reduced recombination around rearrangements and the impact this can have on sequence evolution, specifically the accumulation of genetic incompatibilities. The huntsman spider *Delena cancerides* is known to be highly chromosomally variable, and to have reduced recombination near fusions. However, this species has previously only been interpreted with reference to the classical models of chromosomal speciation, the expectations of which it does not fit well. Broad-scale sampling of this spider has revealed extensive chromosomal diversity and complexity. Twenty one chromosomally differentiated populations (karyomorphs) of this spider have now been described, including those with the putatively ancestral configuration of all telocentric bivalents at meiosis (tII), and many that are saturated for Robertsonian fusions. These include up to six different karyomorphs with metacentric bivalents (mII), eight karyomorphs that form a chain of chromosomes at male meiosis, and six karyomorphs that form two separate but co-segregating chains. A computer simulation was used to test hypotheses regarding the evolution of this chromosomal diversity, which indicated that fusions are likely to have accumulated gradually, possibly due to meiotic drive. Historical phylogeographic analyses have shown that deep cryptic divisions exist which are concordant with the chromosomal diversity. Hybridization experiments have suggested that many hybrid zones between karyomorphs of this species are tension zones, and that genetic incompatibilities are likely to play an important role in generating partial reproductive isolation of karyomorphs. Furthermore, several hybrid zones appear to have been modified by staggered clines. The staggering of clines is thought to ameliorate reproductive isolation mechanisms that are dependent on epistatic fitness interactions, and so may prevent diverging populations progressing towards speciation. Therefore, on the basis of the available evidence, *D. cancerides* may fit the recombination suppression model of chromosomal speciation, although it may be unlikely that the karyomorphs will progress towards full species status. Hence, this species may in the future make a highly informative model organism for investigating the early stages of genetic reproductive isolation associated with chromosomal rearrangements.

Table of Contents

Preface

Style and language

The data chapters in this thesis are presented in the style of published or publishable papers. I have chosen to not write a formal introductory chapter as background material is included in the introduction of each individual chapter, and Chapter 1 is written for a broad audience so as to provide a generalised background for the remaining chapters. It is acknowledged that Chapter 4 is too large to be published as a single paper, but to split this material into smaller units would have required a great deal of repetition which seemed inappropriate for the purposes of thesis examination.

The terminology of cytology and speciation theory has evolved considerably over the years. Below is a brief explanation of the language I have chosen to use.

Telocentric. Following Rowell (1985; 1987b; 1990; 1991a; 1991b), the single armed chromosomes of this species are referred to as telocentric chromosomes, although others have argued that the term 'acrocentric' should be used. While the possibility of short second chromosome arms has never been specifically investigated, in Giemsa stained meiotic spreads centromeres and telomeres do appear to be coincident terminal structures.

Karyomorph. This term has been used throughout to describe a population which appears to be fixed for a specific meiotic chromosomal configuration. This word was chosen in order to avoid the term 'race,' as it is uncertain how the polytypism in this species equates to the definition of chromosomal races used for other systems. Although not definitively defined, the term karyomorph has been used in this context by others in the field (Reig *et al.* 1980; Zambelli & Vidal-Rioja 1995; Rogatcheva *et al.* 2000; Catanesi *et al.* 2002; Ene 2003; Machado *et al.* 2005).

Karyotype. This term is used to describe a specific meiotic configuration (Reig *et al.* 1980; Rogatcheva *et al.* 2000; Catanesi *et al.* 2002; Ene 2003; Machado *et al.* 2005). Thus, each karyomorph is a population of spiders that share a particular karyotype. However, several karyotypes have been observed in hybrid individuals that do not define a karyomorph.

Fusion sets. When fixed fusion heterozygotes undergo meiotic segregation, two sets of fusions are produced; the odd numbered chromosomes in the chain which include one to three X chromosomes, and the even numbered chromosomes which must always segregate away from the X chromosomes. Because autosomes involved in chains are obliged to segregate either with or away from the X's in this way, they are neo-X

and neo-Y chromosomes. Furthermore, members of each group cannot segregate independently, and so are linked into what have been dubbed an X- or Y-linked fusion set.

Collection site descriptors. Each field site at which spiders were collected was given a number, and the initial/s of the closest town or landmark to that site is used as a prefix to the site number. For example, the 39th collection site was close to the town of Bright, and so is referred to as B(39).

At collection sites, each tree in which spiders were found was numbered, each colony or retreat site in which spiders were found on that tree was numbered, and each individual spider taken was also numbered. Thus, spider B(39)8.2.1 is the first spider caught from the second retreat on tree eight at site B(39). Where no more than one colony or retreat was found on any given tree, the retreat site number was excluded. Thus, B(39)3.5 is the fifth spider taken from the third tree at site B(39).

Content

The huntsman spider *Delena cancerides* (araneae: Sparassidae, Walckenaer) encompasses a number of chromosomally distinct parapatric populations (karyomorphs), including forms with entirely telocentric chromosomes, and others saturated for centric fusions resulting in metacentric chromosomes. Three karyomorphs are known in which fusions are sex-linked, meaning that males are fixed heterozygotes carrying a sex-linked chain of chromosomes of various length, while females carry normal bivalents at meiosis. Chromosomal variation of this sort is rarely tolerated in other species, because the segregation of long chromosome multiples frequently results in gametes with too many or too few chromosomes. The resulting reproductive failure may form the basis for reproductive isolation in many species (King 1993), and so the mechanisms that allow *D. cancerides* to segregate long chromosome chains may have allowed this species to maintain species cohesion despite extensive chromosomal variation over its range (Rowell 1985, 1986, 1987b, 1987a, 1990, 1991a, 1991b; Rowell & Avilés 1995).

During a broad scale survey of this polytypism, eleven new chain carrying forms of this species have been identified. Six of these karyomorphs carry two chains at meiosis, and so show segregation behaviour which is beyond our current understanding of meiotic processes (Sharp & Rowell 2007). In addition, a fifth category of chromosomal configuration was observed, where individuals were found to carry rings of various even numbers of autosomes at meiosis. Unlike the chains seen in other populations, these rings are not sex linked and so are observed as floating polymorphism, probably occurring in females as well as males. This polymorphism is

hypothesized to result from hybridization between differentiated metacentric bivalent forming (mII) karyomorphs homozygous for different fusion combinations.

Sequencing of the mitochondrial gene COI from 80 individuals including some from each known karyomorph has revealed ancient divisions within this species. Average corrected pairwise sequence divergence of 11.8% was found among four clades, which is interpreted as resulting from vicariance events, possibly caused by climate change at the end of the Miocene. Abrupt phylogeographic discontinuities in mtDNA and chromosomes exist between clades, with very little mixing of lineages. Therefore, chromosomal diversification appears to have begun after vicariance, but before secondary contact because, although COI clade distributions encompass several karyomorphs each, clade contact zones are highly concordant with karyomorph contact zones. These breaks occur at karyomorph contact zones across which X chromosome fusions differ, suggesting that such differences are related to reduced female-mediated gene flow across contact zones. This widespread species is morphologically and behaviourally invariable, and shows only minor allelic variation between karyomorphs (Rowell 1990), suggesting that male mediated nuclear gene flow across contact zones is relatively unrestricted. Therefore, a signal of ancient divisions within this species has been preserved in the mtDNA of females, whereas males have maintained species continuity by ensuring nuclear gene flow among lineages. These results suggest that despite deep divergences within this species, evolution has been phyletic rather than cladistic. Ancient mtDNA lineages have remained connected by tension zones that act as semi-permeable barriers to gene flow for neutral or positively selected nuclear alleles via male mediated gene flow.

Hybridization experiments among the karyomorphs have revealed unusual hybrid zone structures and cryptic diversity. The mII karyomorph was found to be comprised of up to six distinct karyomorphs, each being homozygous for different Robertsonian fusions. A hybrid zone between these new mII karyomorphs appears to have a very unusual structure, as the most common mII type is found at the centre of the three-way hybrid zone. Several hybrid zones between chain carrying karyomorphs are also unusual, as they appear to have non-coincident clines for sex-specific sets of fusions. This is due to the presence of a recombinant population in between the staggered clines (Searle 1993). Cline separation may be due to stochastic processes because the distribution of this species is highly patchy; however, several lines of evidence suggest that these clines have separated due to selection against hybrid females and one of the reciprocal hybrid male types. Analysis of the fertility data has revealed that genetic reproductive isolation mechanisms are an important feature of hybrid zones between karyomorphs of this species, although recombination pattern differences and malsegregation are also likely to be important.

In order to test hypotheses about the evolution of the chromosomal diversity and complexity observed in *D. cancerides*, a model was designed to simulate fusion

saturation and fixed sex-linked fusion heterozygosity. The range of chromosomal forms now known from the field, and those produced in hybridization experiments, were compared with the expectations generated by computer modelling. This has shown that the currently accepted catastrophic pan-fusion (CPF) hypothesis (Rowell 1987b) does not explain the observations well. Instead, fusions have probably accumulated gradually, meaning that chains have built up by the successive inclusion of new fusions. Fusion saturation appears to have occurred independently in each of the groups of karyomorphs known to make up mtDNA clades. Introgression of fusions, WART mutations and reticulation of lineages through zonal raciation are all likely to have contributed to the observed diversity. Meiotic drive is likely to have been involved in the fusion saturation observed in this species, as well as in spiders more generally.

These findings suggest that *D. cancerides* may not only be the most chromosomally diverse and complex species known, but also potentially a highly informative model organism for resolving the long standing debate regarding the role of chromosomal change in the speciation process (Coyne & Orr 2004).

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