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Antisymmetric male genitalia in Western Australian populations of *Mecyclothorax punctipennis* (Coleoptera: Carabidae: Moriomorphini)

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Abstract

Western Australian populations of *Mecyclothorax punctipennis* (MacLeay) exhibit chiral polymorphism for male genitalic asymmetry. The plesiomorphic genitalic enantiomorph, wherein the male aedeagal median lobe is left side superior when retracted into the abdomen, is rotated 180° to a right side superior position in 23% of males from Western Australia. Conversely, population samples from eastern Australia are monomorphic for the plesiomorphic left side superior condition. Western Australian population samples are significantly heterogeneous for the percentages of chirally reversed males, with right side superior frequencies ranging 0–58%. Conversely, asymmetry of the *M. punctipennis* female reproductive tract, wherein the apex of the bursa copulatrix is distally expanded toward the right side of the individual, is shown to be monomorphic within the species. Based on the vast disparity in frequencies of left versus right enantiomorphs among populations of Western Australian *M. punctipennis*, we hypothesize that population demographic factors related to very small population size and differential gene sampling via genetic drift could interact to establish populations fixed for the novel form. When such chiral genitalic substitutions are coupled with speciation, subsequent diversification stemming from that common ancestor would result in monophyletic lineages characterized by genitalic inversion. This hypothesis is corroborated by the sporadic occurrences of individual males with chirally inverted genitalia throughout the Carabidae, and the known occurrence of eight carabid taxa — individual species to diverse lineages — that are monomorphically characterized by male genitalic inversion.

Keywords

Antisymmetry; genitalic torsion; male genitalia

Introduction

Genitalic asymmetry has evolved numerous times within the Insecta (Huber et al. 2007) with asymmetries most often concentrated in the male structures. Chiral genitalic

antisymmetry, in which mirrored enantiomorphic forms of male genitalia occur within a single species, represents a specialized form of such asymmetry. Conspecific cooccurrence of reciprocally asymmetric male genitalic enantiomorphs has been reported for Mantodea (Holwell & Herberstein 2010), Hemiptera (Baranowski 1958, Guglielmino & Bückle 2010), Coleoptera (Ahrens and Lago 2008), Trichoptera (Schmid 1980, Botosaneanu & Hyslop 1998), and Lepidoptera (Nupponen 2009). These and other examples within these orders have been hypothesized to be the result of sexual selection, either via sexually antagonistic evolution when novel males override female control of mating, or by cryptic female choice when the rarer male genitalic configuration is favored by females (Schilthuizen 2007, 2013).

Across Carabidae, under the plesiomorphic condition of aedeagal asymmetry, the ventral surface of the aedeagal median lobe at rest (i.e., retracted within the abdominal apex) becomes the eudextral surface during copulation. When everted for mating, the tip of the median lobe of typical asymmetrical male genitalia everts to the left side of the abdominal apex, and the median lobe and parameres rotate 90° counterclockwise (Jeannel 1941) to facilitate copulation in the male-above symmetric mating position used by carabid beetles (terminology of Huber et al. 2007). At eversion from the abdomen, the aedeagal parameres articulate with the ventral surface of the median lobe. This everted aedeagal orientation is itself the evolutionary result of 180° torsion of the coleopteran male terminalia from the plesiomorphic state wherein the everted position of the aedeagal assembly places the parameral articulations dorsad (Sharp and Muir 1912, Jeannel 1941). This plesiomorphic “parameres-up” configuration is associated with the ground-plan coleopteran end-to-end mating position (Huber et al. 2007). Parameres associated with the asymmetrically oriented carabid aedeagal median lobe may be differentially developed in length, breadth, shape (Sharp & Muir 1912), or setation when setae are present. Thus, within carabid higher taxa — subfamilial or tribal categories — male genitalia will exhibit a characteristic left and right parameral configuration (Jeannel 1955).

The plesiomorphic left-everting aedeagal configuration has transformed eight different times across Carabidae to the right-everting, right side superior configuration. These instances implicate anywhere from individual species monomorphically characterized by inverted male genitalia, to entire lineages of several hundred species so transformed. Within the tribe Broscini, *Promecoderus* Dejean includes species that represent both configurations (Roig-Juñent 2000), though phylogenetic relationships among *Promecoderus* species remain unstudied. The subtribe Drimostomatina of tribe Cratocerini (sensu Lorenz 2005) — comprising 29 genera and nearly 300 species — is characterized, with the exception of the questionably placed *Diceromerus* (Tschitscherine), by the right side superior male aedeagal configuration (Jeannel 1955). Also within the Pterostichinae, a clade of New Caledonian *Cerabilia* Laporte de Castelnau is characterized by eight species exhibiting right side superior male genitalia (Will unpubl. data), whereas the remainder of the genus distributed in New Caledonia, New Zealand and Australia retains the plesiomorphic left side superior condition. Within the tribe Sphodrini, the right side superior aedeagal condition has evolved independently in the genus *Pristosia* Motschulsky and the species *Calathus ovipennis*

Putzeys (Lindroth 1956). The remainder of Pterostichinae exhibit the plesiomorphic left side superior condition. The subfamily Ctenodactylinae, comprising 19 genera and 131 species of the tribes Ctenodactylini and Hexagoniini (Lorenz 2005), represents an amphi-Atlantic taxon with right side superior male genitalia (Jeannel 1948, 1955). And finally, twice within the Moriormorphini the male aedeagus has transformed to assume the right side superior and so right-everting configuration in: (1) the single Queensland species *Mecyclothorax storeyi* Moore (1984) versus the remainder of species in the genus (Moore 1963); and (2) all species of the Chilean genus *Tropopterus* Solier (Liebherr 2011).

Below we describe the occurrence of chiral antisymmetry within *Mecyclothorax punctipennis* (MacLeay), an extremely common and geographically widespread Australian carabid beetle species (Moore 1984). Populations of *M. punctipennis* in Western Australia polymorphically include males with genitalia representing the plesiomorphic, left side superior as well as the apomorphic right side superior enantiomorph. Specimens representing Western Australian populations of this species vary significantly in the proportions of the two enantiomorphs. Also, based on a sample of over 700 individual males, this genitalic antisymmetry is restricted to Western Australian populations, and does not occur in the eastern Australian populations. Based on dissection of females from a Western Australia population that includes a mix of male enantiomorphs, we show with statistical significance that female reproductive tracts of that population are monomorphic for bursal asymmetry observed across the entire geographic range of the species, thereby demonstrating that female reproductive tract asymmetry is not associated with male chiral genitalic antisymmetry within this species. Finally, we discuss how our observed results can best explain the evolutionary origin of infraspecific genitalic antisymmetry, as well as the numerous instances of species- or clade-specific inverted genitalic asymmetry observed across Carabidae.

Materials and methods

The genesis of this project involved the examination of specimens of *M. punctipennis* in the Western Australian Museum. Within a short series of 10 specimens, two males with antisymmetric genitalic configurations were visible (Fig. 1). Subsequently, material was studied from the Cornell University Insect Collection (CUIC), Ithaca, NY; Essig Museum of Entomology (EMEC), University of California, Berkeley; Field Museum of Natural History (FMNH), Chicago, IL; Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, MA; Queensland Museum (QM), Brisbane; and Western Australian Museum (WAM), Welshpool. Specimens were sorted to locality, with adjacent dates of collecting from those localities grouped together as population samples (Appendix A). Specimens were sexed, and males examined for genitalic asymmetry. Males with the aedeagal median lobe tip everted due to preservation in ethanol (many of the FMNH specimens), were easily scored for genitalic asymmetry. Other teneral or not fully melanized males could be scored by viewing the aedeagal median lobe through the translucent to transparent cuticle of the apical abdominal



Fig. 1. Example males of *M. punctipennis* with partially everted aedeagi (arrows) documenting the apomorphic, right side superior aedeagus (left specimen) and plesiomorphic, left side superior aedeagus (right specimen). Each is photographed over their respective date-locality labels (dissections WA1 and WA2; WAM). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1876312x>.

ventrite. When the median lobe orientation could not be scored, the male specimens were relaxed in near boiling water and the aedeagus was viewed by separating the apical visible abdominal ventrite and tergite with a minuten nadeln probe, or by slightly everting the aedeagal median lobe with a hooked minuten. In order to determine the extent of anatomical asymmetry between left-everting and right-everting males, alcohol-preserved specimens of both forms were dissected and the configuration of their internal structures compared.

Whether the frequencies of plesiomorphic versus inverted male individuals varied significantly among the Western Australian population samples was tested using a replicated goodness of fit (G -statistic) test (Sokal & Rohlf 1981), with results evaluated using the χ^2 distribution (Rohlf & Sokal 1981).

Male genitalic dissections were made for eight specimens each from the Australian states or territories of Western Australia, South Australia, Victoria, New South Wales, Australian Capital Territory and Queensland, plus four dissections of males from Tasmania, in order to assess genitalic variability across the species range. All dissected median lobes and associated parameres, and the laterotergite and antecostal margin of segment IX (Deuve 1993) were photographed in glycerine on a covered microscope slide using a Visionary Digital macrophotography setup with Nikon D1X camera, fibre-optic strobed light source connected to a transmissible light stage, and a Microptics K series lens. Large format 20×39 cm prints of the photographs were compared to assess genitalic conformations among the dissected individuals.

In previously observed female dissections from eastern Australian populations (unpubl. data) an asymmetrical female bursa copulatrix with a distal expansion toward the right side of the beetle had been consistently observed in all individuals. The degree of expansion of the membranous bursal wall varies among individuals, but an expansion toward the right side of the body was a consistent feature. Whether females of Western Australian populations of *M. punctipennis* have compensatory changes in the bursa, e.g., a left side bursal expansion potentially correlated with male enantiomorphs, was investigated using a large series of 244 specimens from Stirling Range N. P., 83 km NE Albany (Appendix A). In this series, 72 males exhibited the plesiomorphic left side superior genitalic condition, and 28 were right side superior. Whether females also exhibited a 28% frequency of left side bursa expansion was tested using a 2×2 contingency test (Graph Pad Software 2014). Given the observed 72 versus 28 frequencies for males, the female column was loaded with various values for the plesiomorphic tract condition, and a 0 value for the hypothesized left bursal expansion. Based on these preliminary tests we established the numbers of plesiomorphic female tracts that we needed to observe, without observing any with the alternative condition, in order to reject at various levels of significance the null hypothesis of identical distributions of right- and left-sided individuals in both sexes. We settled on a female value of 20 plesiomorphic individuals paired with 0 individuals with the alternative condition for our test — null hypothesis rejected at $p = 0.0028$ level of significance (one-tailed test) — and then proceeded to dissect 20 female individuals. During dissection the bursa copulatrix, spermatheca and spermathecal gland were carefully cleared of appended fat body and tracheae, though not twisted nor tamped with dissection tools so that the left-right conformation of the bursa was determined solely by compression of the reproductive tract under the microscope slide cover slip. All female dissections were photographed while in glycerine using the same equipment used to photograph the male dissections, with those photos documenting the results of the test.

Internal soft tissue structures in males, e.g., accessory glands and testes, were examined in four males from Western Australia and four from eastern Australia (New South Wales and South Australia). Ethanol preserved males, two each of both enantiomorphs from Western Australia were selected and their abdomens dissected to determine the number of testes, shape and orientation of the accessory glands and general visceral packing relative to the position of the genitalia.

Male and female dissections were individually numbered, with labels identifying the dissection number added to the specimens so that they may be retrieved from the taxonomic material. Locality and repository data sufficient to identify individual specimens are presented in the figure legends of documenting photographs (Figs. 2, 3 and 5).

Results

When the specimens with partially everted aedeagal apices indicating a right side superior median lobe (Fig. 1) were dissected (Fig. 2) the morphological rotation of male terminalia is shown to involve: (1) the aedeagal median lobe and associated parameres;

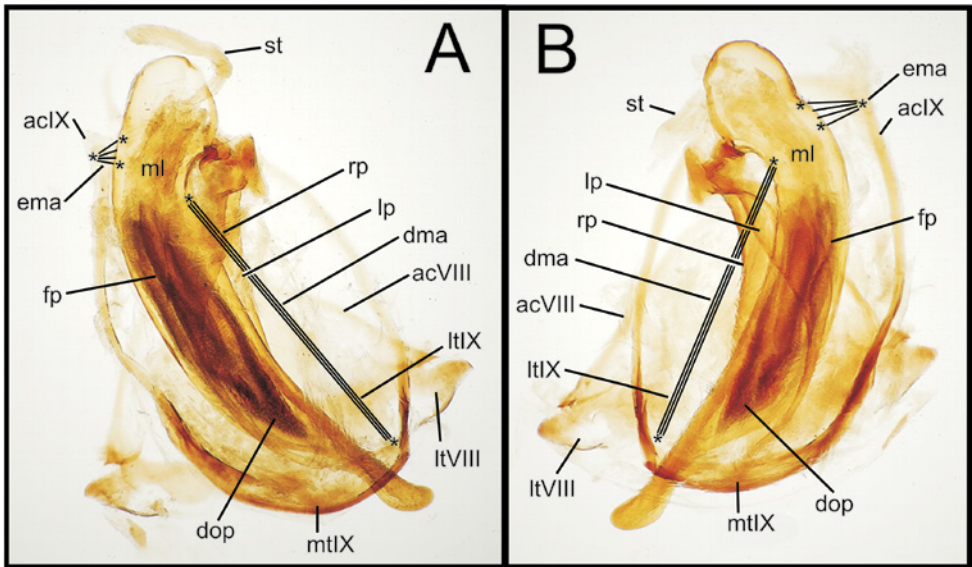


Fig. 2. Dissected male genitalia of *M. punctipennis*, dorsal view (WAM) in their positions of repose with parameres situated dorsally and ventrally. A. Right side superior genitalic enantiomorph (dissection WA1). B. Left side superior genitalic enantiomorph (dissection WA2). In either configuration, the aedeagus is everted by contraction of the depressor muscle of the aedeagus (Iuga & Roşca 1966), which pulls the aedeagal shaft outward at the same time rotating it so that the parameres assume their lateral positions. After mating, the aedeagus is returned to lie within the abdomen by the elevator muscle of the aedeagus, which spans the membrane surrounding the aedeagal base and the apex of the antecostal margin of segment IX. Abbreviations: acVIII, antecostal margin of abdominal segment VIII; acIX, antecostal margin of abdominal segment IX; dma, depressor muscle of aedeagus; dop, dorsal ostial microtrichial plate of aedeagal internal sac; ema, elevator muscle of aedeagus; fp, flagellar plate of internal sac; lp, left paramere; ltVIII, laterotergite of abdominal segment VIII; ltIX, laterotergite of abdominal segment IX; ml, median lobe of aedeagus; mtIX, mediotergite of abdominal segment IX; rp, right paramere; st, sperm tube. This figure is published in colour in the online edition of this journal, which can be accessed via [http://books andjournals.brillonline.com/content/journals/1876312x](http://books.andjournals.brillonline.com/content/journals/1876312x).

(2) the mediotergite, right and left laterotergites and antecostal margin of abdominal segment IX; and (3) the right and left laterotergites and antecostal margin of abdominal segment VIII (Deuve 1993). Abdominal segments VIII and IX are similarly distorted, with their basal extensions lying on the left side of the body in right side superior individuals (Fig. 2A), and on the right side of the body of left side superior individuals (Fig. 2B). The aedeagal median lobe is tubular, and includes an internal sac that is everted during copulation. This sac is distended from a membranous portion of the median lobe apex — the ostium — with the ostium situated on the ventral surface of the median lobe apex (e.g., Fig. 3E). When the internal sac is everted inside the female bursa copulatrix, the sac deploys to the right in the plesiomorphic left side superior males, and to the left in the derived right side superior males. Near its base, the internal sac bears a field of basally directed spicules — the dorsal ostial microtrichial patch (Fig. 3E) — which likely serves as a stickfast device during copulation, as the spicules

are directed posteriorly toward the male when the aedeagal tip has entered the female. Based on male genitalic asymmetry, these spicules would interact with the right side of the female tract when the male is of the left side superior enantiomorph, and with the left side of the female tract in the contralateral male configuration.

The series of eight males dissected to examine the soft tissue organs of the abdomen were uniform in having two testes, a long coiled epididymis, and the gut lying centrally with a single clockwise twist. All specimens had S-shaped accessory glands that varied in their orientation. All four eastern and one western specimen that were left side superior and one western specimen that was right side superior had the accessory gland apices oriented to the right (normal S shape) in both glands. A third western specimen, with aedeagus left side superior, had one accessory gland with the tip oriented right and one oriented left (backward S shape). The fourth western specimen, with aedeagus right side superior, had one accessory gland with the tip oriented right and one with the tip oriented left. The visceral contents of the abdomens were typical of what is seen in carabid beetles (Will et al. 2005) with the only noticeable difference between right and left side superior enantiomorphs being the shift of some organs to accommodate the base of the aedeagus, i.e., the epididymis coil was placed to the contralateral side and the defensive gland reservoir on the side bearing the aedeagal base was situated further laterally and anteriorly. There is no indication of any rotation of internal viscera associated with the alternate genitalic enantiomorphs.

Taking into account the mirrored asymmetries introduced by the presence of left and right male genitalic enantiomorphs in western Australian *M. punctipennis*, the range of shapes observed for the median lobe apex (Fig. 3) do not differ from those presented by Moore (1984), thereby corroborating Moore's circumscription of the species. The principal form of aedeagal variation involves broadening of the median lobe apex in individuals of larger body size; among dissected males, the two largest specimens — one from Western Australia and the other from Queensland — each exhibit a broad median lobe apex (Figs. 3C, J). Moore (1984) illustrated a third male from South Australia exhibiting a broad aedeagus, suggesting that this less common genitalic conformation occurs across the geographic range of the species.

Frequency distributions of left and right aedeagal forms for *M. punctipennis* vary dramatically across the species range (Fig. 4). All collecting series examined from eastern Australia monomorphically comprise the pleisomorphic left side superior enantiomorph. Right side superior male genitalia are observed only in populations from Western Australia, though their frequency in collecting series ranges 0–58%. Collecting series from West Cape Howe generally contain around 20% right side superior individuals, though a series of 15 males from the Giant Tingle tree area, Walpole N. P. are monomorphically left side superior. Three population samples from the Perth area include two samples with 14–15% right side superior individuals, contrasted with a series of 15 from Heirisson Is. that are monomorphically left side superior. The enantiomorphic distributions for the 19 collecting series from Western Australia exhibit statistically significant frequency independence ($\chi^2 = 23.59$, $df = 18$, $p < 0.005$).

Western Australian female *M. punctipennis* do not exhibit genitalic inversion based on the results from 20 female dissections (Fig. 5). All female dissections show a distal

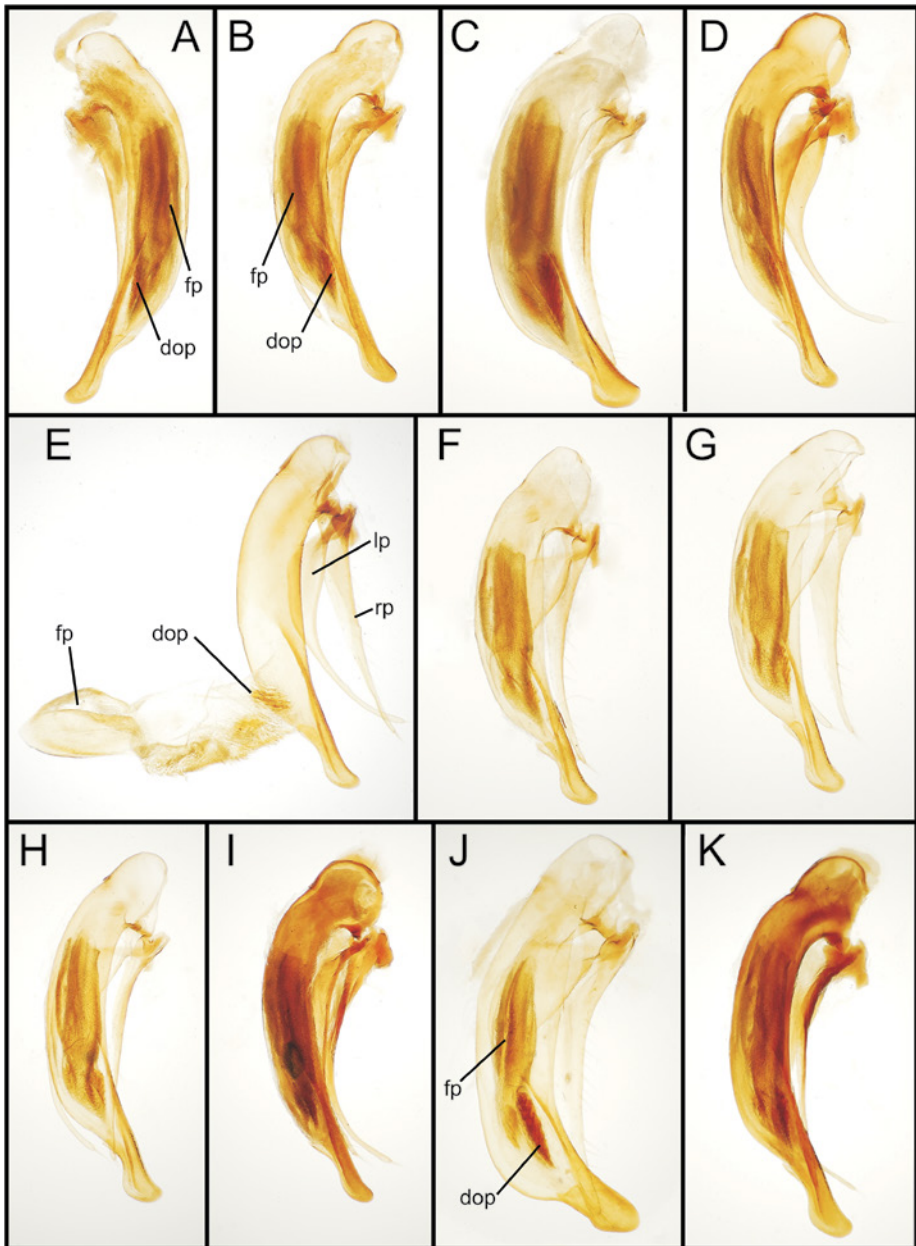


Fig. 3. Representative dissected male aedeagal median lobes and associated parameres of *M. punctipennis* from localities across the species' range; ventral view when aedeagus in repose, all images at equal scale of magnification (male dissection number; repository). Abbreviations as in Fig. 2. A. WA: N. side Torndirrup N.P. (WA1; WAM). B. WA: Stirling Rge. N.P. (WA2; WAM). C. WA: 85 km N Albany (WA4; FMNH). D. SA: 1.5 km N Yunta (SA2; FMNH). E. VIC: Benella (VIC2; CUIC). F. VIC: Mt. Buffalo N.P. (VIC5; CUIC). G. NSW: Kosciuszko N.P. (NSW2; CUIC). H. ACT: Black Mountain (ACT8; CUIC). I. QLD: Blackdown Tbl. (QLD1; QM). J. QLD: Mt. Tambourine (QLD6; MCZ). K. TAS: Hobart (TAS2; MCZ). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1876312x>.

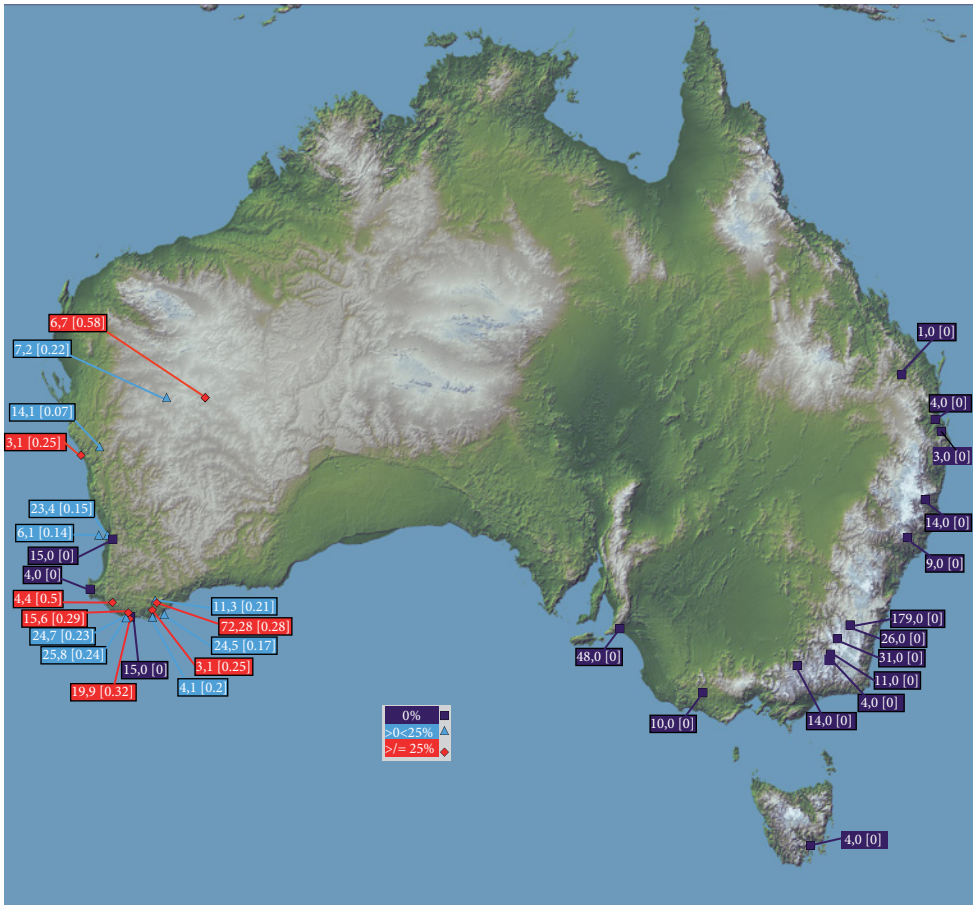


Fig. 4. Geographic distribution of *M. punctipennis* population samples (Appendix a); boxes associated with localities include number of left superior males, number of right superior males (and fraction right superior males in brackets). Base map taken from NASA/JPL/NGA (2004); green coloration denotes lower elevations, white denotes highest elevations. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1876312x>.

expansion or curvature of the bursa copulatrix toward the right side of the individual, indicating that the frequency distribution for female genitalic orientation is independent from that observed for male genitalia ($p = 0.0028$, one-tailed test).

Discussion

Left-right asymmetry in *Drosophila* (Diptera: Drosophilidae) has been shown to be under control of the myosin 31DF gene, with one mutant reported to reverse orientation of the visceral organs (Hozumi et al. 2006), and a second controlling the direction of torsion in the genitalia (Spéder et al. 2006). In both instances, the wild type myosin allele is dominant to the mirror-image mutant. If left-right male genitalic orientation

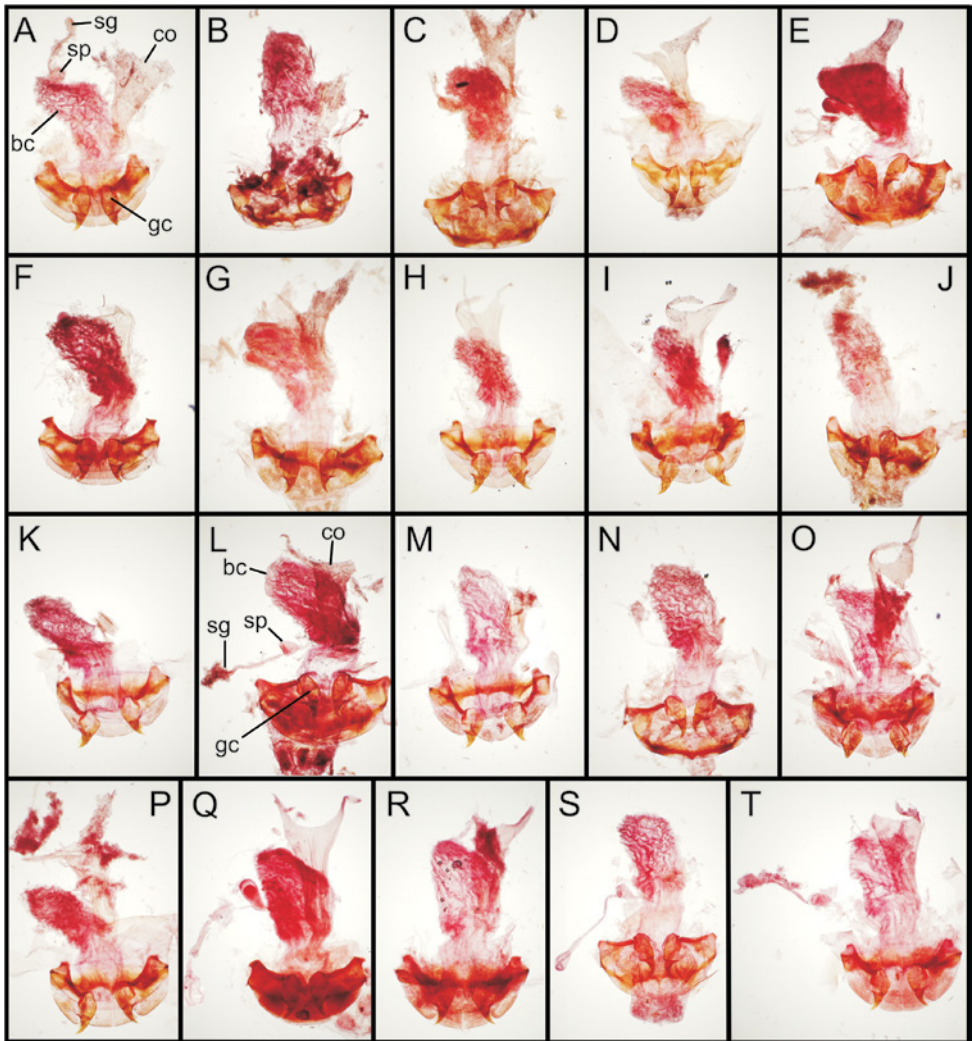


Fig. 5. Dissected female reproductive tracts for 20 individuals from WA: 83 km NE Albany, Stirling Rge. N.P.; ventral view, all images at equal scale of magnification (WA female dissections 1–20; FMNH). Structures labeled in panels A and L: bc, bursa copulatrix; co, common oviduct; gc, gonocoxite; sg, spermathecal gland; sp, spermatheca. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1876312x>.

in *M. punctipennis* were to follow this model, then a single mutant allele would govern development of the apomorphic right side superior enantiomorph that implicates abdominal segments VIII and IX plus the aedeagus. Deuve (1992) demonstrated, through examination of a left-right gynandromorph of *Cotinus mutabilis* Gory & Percheron (Coleoptera: Scarabaeidae), that the male aedeagus is derived from abdominal segment X, whereas the female external genitalia — the gonocoxae or ovipositor — are derived from abdominal segment IX. Based on topological concordance and an

assumption that a membranous pouch on the female half of the *Cotinus* specimen represents the bursa copulatrix, Deuve (1992) concluded that both the bursa and the male aedeagus are associable with segment X. Nevertheless, the bursa copulatrix in Western Australian *M. punctipennis* is monomorphically oriented toward the right side in females, though the males exhibit both genitalic enantiomorphs. Thus if male genitalic orientation is governed by alternate myosin alleles, those alleles cause reversed genitalic orientation in male segments VIII–X but do not influence development of the female bursa copulatrix hypothesized to form part of segment X. The mechanism of sex determination is not known for Moriomorphini, though related taxa within the supertribe Trechitae may be XX female, XO male, or in the case of *Bembidion* Latreille — predominantly XY male (Maddison 1985). Thus at present we are unable to hypothesize how sex linkage with developmental genes might operate in determining male genitalic orientation. Independence of male and female genitalic asymmetry has been reported in a converse example, wherein females of the Hawaiian *Blackburnia incendiaria* (Blackburn) exhibit inverted asymmetrical bursae in contrast to its sister species *B. rupicola* (Blackburn), whereas males of both species are monomorphic for the plesiomorphic, left side superior aedeagus (Liebherr & Zimmerman 2000). Thus evidence at hand for Carabidae suggests independent evolution of genitalic asymmetry in males versus females.

Schilthuizen (2007) argued that insect genitalia are under strong sexual selection pressure, and so genitalic antisymmetry should be an evolutionarily unstable situation. If a species exhibits antisymmetry in male genitalia, the rare form of male may be favored if females prefer novel males. Such a situation would result in negative frequency-dependent selection (Hughes et al. 2013), and for chiral antisymmetry, left-right male ratios would hover near 1:1; a condition termed pure antisymmetry (Schilthuizen 2013). Frequency-dependent selection appears to operate in the snail subgenus *Amphidromus* (Mollusca: Camaenidae) which includes predominantly antisymmetric species. In these species dextral and sinistral individuals mate more frequently than expected by chance and spermatophores of one chiral form align best with the juncture of the spermatophore receiving organ and the oviduct of the opposite chiral form, presumably enhancing fecundity (Schilthuizen et al. 2007). Thus the predominance and relative fitness advantages of interchiral matings establish a stable antisymmetric condition within these species.

Under an alternate sexual selection hypothesis (Schilthuizen 2013), females' control of sperm transfer via the blocking of unwanted male intromission could be overcome by alternate enantiomorphic males, thereby antagonistically driving their novel gene to fixation in the species. However, the observed frequencies of genitalic enantiomorphs in western populations of *M. punctipennis* do not adhere to an average of 1:1 as predicted by a rare male hypothesis, nor do they approach fixation of the novel form, as predicted by antagonistic male evolution. Whereas the apomorphic enantiomorph accounts for an average of 23% of the male individuals over all western populations, the various population samples include significantly heterogeneous frequencies of such enantiomorphs, ranging from 0–58%. This heterogeneity suggests that gene flow is not sufficient to overcome local factors underlying populational divergence of this

condition. That lack of gene flow has some influence in dictating frequencies of the apomorphically rotated genitalic form is shown by the absence of the novel enantiomorph in eastern Australia. Thus we conclude that the deserts of the Nullarbor Plain currently act as a barrier disjunctly isolating western from eastern Australian populations of *M. punctipennis*.

The present data do not illustrate any correlation between genitalic inversion frequency and geographic proximity of population samples. Taking 1976–1977 samples (FMNH) made along the southern coast of Western Australia (Appendix 1), there are five neighboring population samples from Walpole-Nornalup National Park and vicinity with male inversion frequencies disparately spanning 0, 0.23, 0.24, 0.29 and 0.32 (Fig. 4). Conversely, five population samples taken from the east near West Cape Howe (Stirling Range and Porongurup N.P., Cheynes Beach, and Two People's Bay) reflect more uniform frequencies of 0.17, 0.20, 0.21, 0.25 and 0.28 male inversion. We propose that population demographic factors may differentially sort the local distributions of genitalic forms, with small effective population sizes and genetic drift contributing to the observed mosaic of genitalic frequencies. Based on the moderate frequencies of inverted males in Western Australian populations, a colonization event involving only a few beetles could establish a population fixed or significantly skewed toward fixation of either morph. Thus neighboring populations may over- or under-represent the rarer inverted form. This scenario requires little or no sexual selection for or against novel males. Such near-neutrality among genitalic forms appears to fit the pattern presented by *Ciulfina* praying mantids in Queensland, wherein three of the five species exhibit male genitalic antisymmetry, and geographically adjacent populations within species may vary dramatically in left-right enantiomorphic frequencies (Holwell and Herberstein 2010). Moreover, experimental interspecific matings of *Ciulfina* individuals with opposite genitalic enantiomorphs did not result in any premating reproductive isolation, though postmating reproductive isolation between species was observed.

That mirrored genitalic forms of Western Australian male *M. punctipennis* cohabit with females monomorphically exhibiting a rightwardly expanded bursa copulatrix raises the question of how male and female genitalia interact during mating among individuals of these populations. The internal sac of the plesiomorphic left side superior males deploys from the right side of the median lobe apex during copulation, in line with the rightward extension of the female bursa copulatrix. Right side superior males, conversely, have the internal sac deployed from the left apical surface of the median lobe, apparently requiring a sinuous “right-hand turn” in order for the apex of the sac to enter the bursal extension. However in *Mecyclothorax* spp. sperm is transferred from male to female while held inside a spermatophore comprising an apical sperm ampulla and a basal collar and stem (Liebherr 2013, Fig. 4). This spermatophore cannot enter the spermathecal duct as its apical diameter is approximately 0.25 mm, 10× the diameter of the spermathecal duct. Thus sperm are released from the spermatophore to travel up the spermathecal duct while the spermatophore is held inside the female bursa copulatrix. Because the juncture of the spermathecal duct and the bursal wall lies along the dorsal midline (Liebherr & Will 1998), it appears that

spermatophores produced by males of both chiral genitalic enantiomorphs are able to pass sperm into the spermathecal duct. Moreover, because the female bursa copulatrix is also integral to fertilization and oviposition, the configuration of the female tract may be under the influence of selective forces far beyond those related to copulation or the transfer of sperm to the spermatheca.

The hypothesis that novel genitalic alleles can become fixed in small, isolated populations of an antisymmetric species requires several conditions related both to the origin of such mutants, and to the likely speciation process. That rare mirror-image genitalic enantiomorphs exist within carabid species otherwise characterized by plesiomorphically oriented male genitalia has been documented sporadically, suggesting that the phenomenon is taxonomically widespread. Jeannel (1942) reported an inverted male of the widespread Palaearctic *Ophonus rufipes* (DeGeer) (tribe Harpalini) from Finistère, France. Recently an inverted male of a cryptic species in the *Bembidion kuprianovi* complex (tribe Bembidiini) was discovered (Maddison 2013). Thus alternate enantiomorphs appear to occur commonly enough that taxonomists conducting genitalia dissections occasionally discover them (Fig. 1). At the other end of the evolutionary time scale reside those species or multi-species lineages characterized by apomorphically inverted genitalia. Within the tribe Moriormorphini, *Mecyclothorax storeyi*, a species restricted to Mt. Bellenden Ker in the Queensland Alps, monomorphically includes males with inverted genitalia. Among the approximately 20 Australian *Mecyclothorax* species (Moore et al. 1987, Baehr 2003, 2009), *M. punctipennis* and *M. storeyi* are not closely related (Moore 1984), establishing independent evolutionary origins for their inverted male genitalia. Additionally, males of all species in the Austral-disjunct genus *Tropopterus*, the tribe's sole South American representative taxon, possess inverted genitalia. *Tropopterus* and *Mecyclothorax* are, respectively, member taxa of the two moriomorphine subtribes Moriormorphina and Amblytelina (Liebherr 2011), again pointing to evolutionary independence of these genitalic inversion events. Cladistic placement of *Tropopterus* within Moriormorphini is inconsistent with Gondwanan vicariance, as the entire tribe exhibits repeated Australia-New Zealand sister-area relationships; one such set of taxa diverging prior to the origin of *Tropopterus* (Liebherr 2011). Rather it appears that the biogeographic history of *Tropopterus* is consistent with a southern dispersal pattern (San Martín & Ronquist 2004). Both examples of genitalic inversion within Moriormorphini — *M. storeyi* on Mt. Bellenden Ker, and *Tropopterus* in Chile — suggest a taxon's founding by small numbers of individuals in isolated locations, consistent with the founders' inheriting a limited genetic legacy fixed for independently evolved alleles coding for inverted male genitalia.

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Appendix A. Genitalic enantiomorphic data from population samples of *Mecyclothorax punctipennis* (MacLeay); no. left refers to number of plesiomorphic left side superior males, no. right refers to number of apomorphic right side superior males.

State	Locality	Latitude	Longitude	Year	No. males	No. left	No. right	Fraction right	No. females	Collection.
WA	Meekeatharra	S26°35'29"	E118°29'48"	1931	9	7	2	0.22	11	MCZ
WA	Wiluna	S26°35'49"	E120°13'29"	1931	13	6	7	0.58	16	MCZ
WA	Mullewa	S28°32'22"	E115°30'45"	1931	15	14	1	0.07	22	MCZ
WA	Geraldton	S28°46'34"	E114°36'46"	1931	4	3	1	0.25	3	MCZ
WA	Perth, Mt. Lawley	S31°56'15"	E115°51'48"	1977	27	23	4	0.15	48	FMNH
WA	Heirisson Is., Perth	S31°57'57"	E115°52'57"	1953	15	15	0	0	23	MCZ
WA	Roronest Is.	S31°59'49"	E115°32'24"	1931	7	6	1	0.14	6	MCZ
WA	Margaret R.	S33°57'13"	E115°04'25"	1931	4	4	0	0	2	MCZ
WA	Stirling Range N.P., 83 km NE Albany	S34°21'50"	E117°59'20"	1976–1977	100	72	28	0.28	144	FMNH
WA	Stirling Range N.P., Toolbrunup Peak	S34°22'00"	E118°02'57"	1976	14	11	3	0.21	22	FMNH
WA	Pemberton	S34°26'43"	E116°02'03"	1931	8	4	4	0.5	14	MCZ
WA	Porongurup N.P.	S34°40'34"	E117°52'06"	1976	29	24	5	0.17	21	FMNH
WA	Mt. Frankland, 29 km N Walpole	S34°49'27"	E116°47'19"	1976	21	15	6	0.29	34	FMNH
WA	Cheynes Beach, 52 km NE Albany	S34°53'03"	E118°24'17"	1976	4	3	1	0.25	6	FMNH
WA	Walpole-Nornalup N.P., Mt. Clare Cpgd.	S34°58'49"	E116°43'41"	1976	31	24	7	0.23	56	FMNH
WA	Walpole-Nornalup N.P., Giant Tingle Area	S34°59'06"	E116°53'55"	1976	15	15	0	0	38	FMNH
WA	Conspicuous Pt. SE Nornalup	S35°02'54"	E116°50'52"	1976	28	19	9	0.32	36	FMNH
WA	Walpole-Nornalup N.P., Coalbine	S35°00'26"	E116°41'22"	1976	33	25	8	0.24	56	FMNH
WA	Two People's Bay, 43 km E Albany (Mt. Gardner)	S35°58'59"	E118°09'11"	1977	5	4	1	0.2	10	FMNH
SA	Tookyerla Ck., Currency Creek Winery	S35°24'48"	E138°47'33"	2011	48	48	0	0	84	CUIC
VIC	Lake Carani, Mt. Buffalo N.P.	S36°44'09"	E146°43'45"	2011	14	14	0	0	24	CUIC
VIC	Cookaburra Station, Glenthompson	S37°42'23"	E142°31'27"	2011	10	10	0	0	14	CUIC
TAS	Hobart	S42°52'50"	E147°19'30"	1956–1957	4	4	0	0	4	MCZ
ACT	Black Mountain	S35°16'24"	E149°06'22"	1991	179	179	0	0	213	CUIC
ACT	Black Mountain	S35°16'24"	E149°06'22"	1999	26	26	0	0	29	CUIC
NSW	New England N.P.	S30°34'59"	E152°28'30"	1957	14	14	0	0	13	MCZ

NSW	Barrington Tops	S32°01'42"	E151°39'48"	1932	9	9	0	0	7	MCZ
NSW	Kosciuszko N.P., Bullock Hill Tr.	S35°46'35"	E148°31'30"	2011	31	31	0	0	32	CUJC
NSW	Kosciuszko N.P., Grass Flat Ck.	S36°19'54"	E148°14'05"	1999	11	11	0	0	11	CUJC
NSW	Kosciuszko N.P., Leatherbarrel Ck.	S36°31'32"	E148°11'36"	1999	4	4	0	0	3	CUJC
QLD	Nipping Gully	S25°41'00"	E151°25'05"	1999	1	1	0	0	1	QM
QLD	vic. Brisbane	S27°28'05"	E152°55'07"	1943	4	4	0	0	9	MCZ
QLD	Mt. Tambourine	S27°55'39"	E153°11'39"	1943	3	3	0	0	4	MCZ

See Materials and methods for institutional collection codes.