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Fitness advantages of the biased use of paired laterally symmetrical penises in an insect

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Abstract

The evolution of laterality, that is the biased use of laterally paired, morphologically symmetrical organs, has attracted the interest of researchers from a variety of disciplines. It is, however, difficult to quantify the fitness benefits of laterality because many organs, such as human hands, possess multimodal functions. Males of the earwig *Labidura riparia* (Insecta: Dermaptera: Labiduridae) have morphologically similar laterally paired penises, only one of which is used for inseminating the female during a single copulation bout, and thus provide a rare opportunity to address how selection pressure may shape the evolution of population-level laterality. Our population studies revealed that in 10 populations, located at 2.23–43.3° north, the right penis is predominantly used for copulating (88.6%). A damaged penis was found in 23% of rare left-handers, suggesting that the left penis can function as a spare when the right one is damaged. By pairing *L. riparia* females with surgically manipulated males, we found that males forced to use the right penis outperformed left-handed males in copulation (the probability of establishing genital coupling during the 1-hr observation period: odds ratio [OR] of 3.50) and insemination (probability of transferring a detectable amount of sperm: OR of 2.94). This right-handed advantage may be due to the coiled morphology of the sperm storage organ with a right-facing opening. Thus, female genital morphology may play a significant role in the evolution of handedness and may have acted as a driving force to reduce penis number in related taxa.

KEYWORDS
evolution of laterality, genital coevolution, morphological asymmetry, sexual conflicts, sexual selection

1 | INTRODUCTION

Humans predominantly use their right hand for many tasks, even though the right and left hands are morphologically symmetrical (Annett, 2013). This type of asymmetry, termed population-level laterality or handedness, is of interest to researchers and the general public. Although most studies of population-level laterality were conducted in laterally paired organs, such as eyes, limbs and fins, of vertebrate taxa (Rogers & Andrew, 2002; Vallortigara, Chiandetti, & Sovrano, 2011; but see also, e.g., Quaranta, Siniscalchi, & Vallortigara, 2007, for cases of laterality in unpaired organs), this phenomenon is also widespread in invertebrates (Frasnelli, Vallortigara, & Rogers, 2012). Especially, with the notable diversity in their life history, physiology and morphology and the ease of maintaining many individuals under laboratory conditions, insects are becoming important model organisms for studying the evolution of population-level laterality (Niven & Bell, 2018; Niven & Frasnelli, 2018; Rogers & Vallortigara, 2015, 2019).
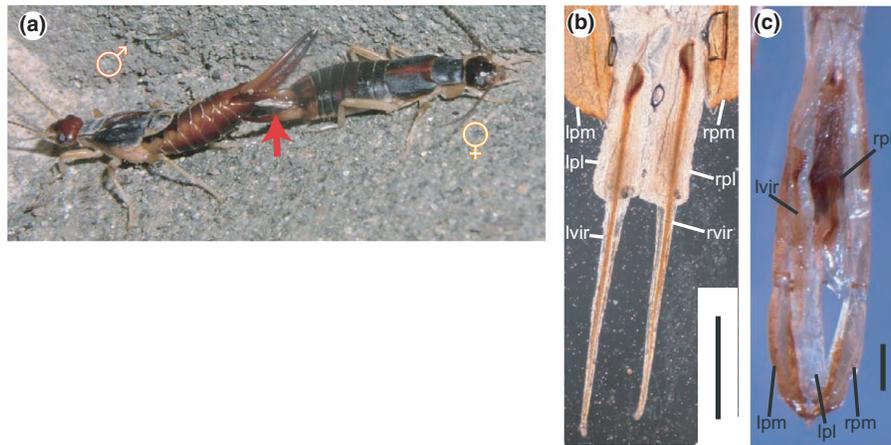
FIGURE 1 Mating posture (a) and male genitalia (b, c) of *Labidura riparia*. (a) A mating pair in the wild (Kumagaya population, Japan). The male genitalia are indicated by the arrow. (b) Genitalia of a Miura-population (Japan) male, showing a case in which no lateral differentiation has been developed: both the left and right virgae, each in the left and right penis lobes, point posteriorly. (c) Genitalia of a Penang-population (Malaysia) male. The left penis is intact and pointing posteriorly, whereas the right one is necrotic and flexed. lpl, left penis lobe; lpm, left paramere; lvir, left virga; rpl, right penis lobe; rpm, right paramere; rvir, right virga. Scale bars: 500 μm

Due to the multimodal functions of many laterally paired organs showing handedness, it is usually difficult to estimate the proximate advantages of lateralization based on laboratory experiments evaluating a particular task. Thus, the costs and benefits of population-level laterality are largely unknown in both vertebrates (but see Rogers, Zucca, & Vallortigara, 2004) and invertebrates (Niven & Frasnelli, 2018). The genitalia of the male earwig, *Labidura riparia* (Pallas, 1773), provides a rare opportunity to quantify the selection pressures that cause the biased use of laterally paired organs. Each of the paired penises of this insect consists of a sclerotized sperm-transferring tube, termed virga, and a surrounding membranous sac, termed penis lobe (Figure 1b; Kamimura, 2006). Although both penises are fully functional with no detectable differences in their morphology, *L. riparia* males predominantly use the right penis for transferring sperm to the female spermatheca, a sperm storage organ (Figure 1b; Kamimura, 2006). This behavioural asymmetry is also apparent when in repose, as the right penis points posteriorly and is thus ready to establish the end-to-end copulation posture (Figure 1a), whereas the left one bends at the base to point anteriorly in the majority of males (Figure 2; Kamimura, 2006). The base of the female spermatheca is spiralled, which may be responsible for the biased use of the right penis (Kamimura & Lee, 2014b). Because the penises are used only for copulation, and the genitalia are completely retracted into the genital chamber when in repose, this organ enables us to estimate selection pressure necessary for the evolution of laterality more accurately than other cases.

Whereas numerous examples involving morphologically asymmetrical genitals in otherwise laterally symmetrical animals exist (Huber, Sinclair, & Schmitt, 2007; Schilthuizen, 2013), to our knowledge, conspicuous laterality of paired genitals has only been reported in earwigs of Labiduridae (Frasnelli et al., 2012; Kamimura, 2006; Kamimura & Lee, 2014b). In earwigs retaining many plesiomorphic characteristics (Diplatyidae, Pygidicranidae and Anisolabididae), males use their right and left penises equally (Kamimura, 2004a; Kamimura & Lee, 2014a;
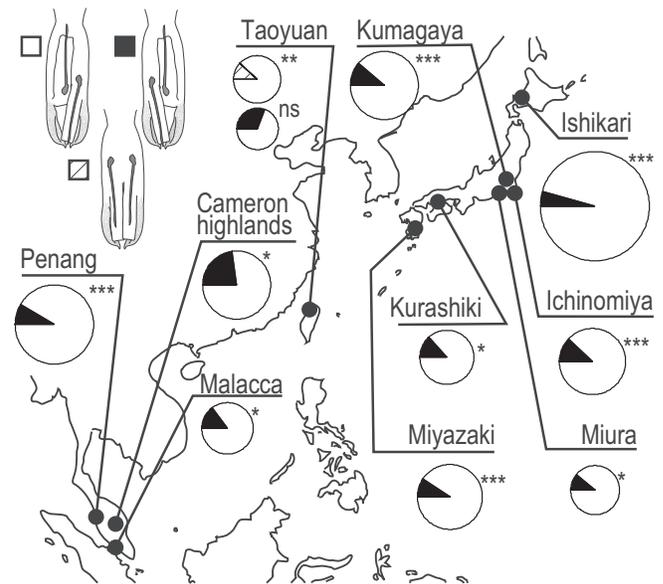

FIGURE 2 Penial laterality of 10 populations of *Labidura riparia* from Malaysia, Taiwan and Japan. Open, filled and hatched parts represent the R-ready (or R-used) males, L-ready (or L-used) males, and males with both penises pointing backwards, respectively. The size of each circle represents the number of males examined (see Table 1). The data for five Japanese populations (Ishikari, Kumagaya, Ichinomiya, Kurashiki and Miyazaki) are from Kamimura (2006). *p < 0.01, **p < 0.001, ***p < 0.0001, after correcting for multiple comparisons using FDR

Kamimura & Matsuo, 2001), whereas the members of the superfamily Forficuloidea (or the nanorder Eudermaptera) possess only the right penis, with complete loss of the left one (Kamimura, 2006). Interestingly, the family Labiduridae, to which *L. riparia* belongs, is considered a sister clade of the Forficuloidea (Colgan, Cassis, & Beacham, 2003; Haas, 1995; Haas & Kukalová-Peck, 2001; Jarvis, Haas, & Whiting, 2005; Kamimura, 2004b; Kocarek, John, & Hulva, 2013; Naegle, Mugleston, Bybee, & Whiting, 2016; Wirth, Guellec, & Veuille, 1999), suggesting a

Locality	North latitude	East longitude	Number of males examined	Source
Ishikari, Japan (IK)	43.26	141.36	90	Kamimura (2006)
Kumagaya, Japan (KG)	36.13	139.38	35	Kamimura (2006)
Ichinomiya, Japan (IM)	35.37	140.38	33	Kamimura (2006)
Miura, Japan (MU)	35.19	139.67	18	This study
Kurashiki, Japan (KS)	34.50	133.64	22	Kamimura (2006)
Miyazaki, Japan (MZ)	31.85	131.45	32	Kamimura (2006)
Taoyuan, Taiwan (TY)	25.12	121.24	17 + 13[a]	This study
Penang Is., Malaysia (PN)	5.35	100.31	47 (19)[b]	This study
Cameron Highlands, Malaysia (CH)	4.50	101.41	35	This study
Malacca, Malaysia (ML)	2.23	102.15	20	This study
Total			362	

[a]Offspring of two wild-caught females. [b]Numbers in the parentheses are those collected as a nymph and reared to adulthood in the laboratory.

transition from behavioural laterality to the eventual evolutionary loss of the less frequently used left penis (Kamimura, 2006; Kamimura & Lee, 2014b; Palmer, 2006). Given the fact that no general explanation has been proposed to date for the evolution of asymmetrical genital morphology in animals (Huber et al., 2007; Schilthuizen, 2013), understanding the evolutionary forces underlying behavioural asymmetries in *L. riparia* may shed light on subsequent morphological evolution, including changes in the body plans of earwigs.

In this study, we quantified the selective advantage of right-handedness in *L. riparia* by surgical manipulation of male genitalia, which mimicked natural penial damage. The causes of differences in reproductive success between right- and left-handed males were also explored by observing male and female genitalia during copulation. Based on the results and a review of previous studies, we compare advantage in fitness components between population-level laterality and morphological asymmetries.

2 | MATERIALS AND METHODS

2.1 | Study populations and male genital handedness

Kamimura (2006) reported consistently right-biased use of paired penises in five Japanese populations of *L. riparia*. Five additional populations from Malaysia (*n* = 3; CH, ML and PN), Taiwan (*n* = 1; TY) and Japan (*n* = 1; MU) were also used in the present study (Table 1). Adults and nymphs were collected by hand mainly during the nighttime. Nymphs were reared to adulthood at 26 ± 1°C under a 12-hr light–dark cycle, as per Kamimura (2006). Offspring of two field-collected TY females were reared separately to adulthood. A larger number of field-collected, unrelated samples were examined from other populations (Table 1). The cultures were examined and food was changed every 3 days. In *L. riparia*, both the right and left penises point backwards just after the imaginal eclosion. One of them, usually the left, subsequently bends to point forward within 6 days after eclosion (Kamimura, 2006). Accordingly, adult samples were fixed by placing them in a refrigerator (–20°C) at least 6 days after imaginal eclosion. Later, the genitalia were dissected out, mounted on a slide with phosphate-buffered saline and observed under a light microscope (Olympus BX53 or CX21, 40–400X). Because the penis should point posteriorly during copulation, organs in this position were considered ready for copulation (scored as R-ready or L-ready for individuals having the right or left penis pointing posteriorly, respectively).

2.2 | Female genitalia

As described by Hudson (1973), the proximal part of the spermatheca of *L. riparia* is a characteristically coiled. Kamimura and Lee (2014b)

reported that the coiling was sinistral (sensu used in gastropods, e.g. Schilthuizen & Davison, 2005) in 62 of 65 Japanese and Malaysian females (95.3%) but ambiguous in the other three.

In this study, the direction of the spermathecal opening was re-examined in the Japanese samples (IK and MZ) in detail. The female reproductive organs (ovaries, oviducts, spermatheca and vagina) were removed from euthanized females and mounted on a slide (n = 25). To prevent conformational changes, the spermatheca was suspended in a small volume of glycerol by fixing the lateral oviducts and post-vagina between the pieces of soft plastic tape used as spacers (T-4610; Nitoms, Inc.; 1 mm in thickness) and a coverslip (Figure S1). Samples were viewed using laser scanning confocal microscopy (LSM-410, Zeiss, or Fluoview FV300; Olympus) without staining, as earwig spermathecae emit strong autofluorescence when excited by UV light (Kamimura & Lee, 2014b). Based on the stacked image of each female, the opening angle of the spermatheca was measured with respect to the anterior–posterior axis of the body, to the nearest 1° using a protractor.

2.3 | Reproductive success of surgically treated males

All of the following experiments were conducted using virgin adults from the Penang (PN) population that were laboratory-raised under the conditions specified above. Males were anaesthetized with carbon dioxide gas and randomly assigned to one of three surgical treatments. In the right-handed (RH) and left-handed (LH) ablation treatments, the left or right penis and interior virga were removed with a pair of fine forceps from a male, effectively forcing the male to be right- or left-handed, respectively. The genitalia of control (Ctl) males were extracted from the genital chamber, but neither penis was ablated. To minimize differences between the RH and LH treated males, the ablation treatment was conducted within 3 days of the imaginal eclosion when both of the penises were still pointing posteriorly.

A virgin male and a virgin female, 6–39 days after the imaginal eclosion (at least 3 days after the surgical treatment), were paired (n = 111 pairs, n = 37 pairs per treatment). Siblings were not paired to avoid possible effects of kin recognition. A time-lapse recording (GZ-MG980S; Victor) of one frame per 2 s was used to record behaviour for 1 hr. To allow for acclimation, the females were released into individual plastic containers (60 mm diameter, 40 mm height) with transparent plastic lids and plaster base 10 min prior to the start of recording. Males were introduced immediately before the trial. Since most copulations of virgin pairs occur during the first hour of paring, irrespective of lighting conditions (Y. Kamimura, unpublished data), behavioural trials began between 15:45 and 17:05, that is before the 19:00 initiation of the 12-hr dark phase. One pair from each treatment group was observed simultaneously in each trial, with 1–2 trials recorded per day. The position of the pairing vessels in the recording box was rotated between trials to control for possible effects of position on behaviour. After recording, the pair was euthanized by placement in a freezer. Insemination status in females was

recorded as "0" (with no detectable sperm in the spermatheca) or "1" (with sperm in the spermatheca), and the prothoracic width in males and females was measured as an index of body size to the nearest 1 pixel (corresponding to 0.00688 mm) using ImageJ software (Collins, 2007). Four trials were excluded from analyses because of cannibalism or dissection failure of the female in at least one pair.

2.4 | Coupling of genitalia during copulation

At 5 min after copulation initiation, copulating pairs (n = 5, 9 and 6 for Ctl, LH and RH, respectively) were fixed with absolute ethanol containing dry ice particles and immediately stored in a freezer (–20°C) to fix for over 2 weeks. Meshed male and female terminalia (the penultimate abdominal segments and beyond) were trimmed and transferred to a BABB solution (1:2 benzyl alcohol:benzyl benzoate) to make muscles and sclerites transparent for in situ examination (Kamimura & Mitsumoto, 2011). Depigmentation of heavily sclerotized sclerites progresses gradually in this solution. After 8–18 months in the clearing solution at 25°C, genital coupling samples were observed and photographed under a differential interference contrast (DIC) microscope (BX53, 100–400X; Olympus). From these photographs, we created a digital composition of in-focus parts selected from each image using the CombineZM image-stacking software (Hadley, 2008).

2.5 | Statistical analysis

All statistical analyses were conducted using R 3.4.1 software (R Core Team, 2017). A binomial test was used for each population, for testing deviations from the null hypothesis that the ratio of R- to L-ready males is 1:1. Significance thresholds in multiple comparisons were corrected using the false discovery rate (FDR; Benjamini & Hochberg, 1995).

To analyse the effects of surgical treatments on male reproductive success, generalized linear mixed models (GLMMs) were fitted using the "glmer()" function in the "lme4" package (Bates, 2005) by incorporating the trial (=block) number as a random factor (randomized complete block design; Montgomery, 2017). Four components of male reproductive success were evaluated: copulation success, insemination success, total copulation duration and latency to the first copulation. For the analysis of the former two dependent variables, we adopted a binomial error structure and a logistic link function. Copulation success was scored as "0" (males that did not copulate) or "1" (males that copulated at least once with apparent genital coupling >1 min). A Gaussian error structure and an identity link function were used for analyses of latency to the first copulation and total copulation duration such that only the cases with at least one copulation were analysed. We adopted a forward variable-selection strategy, starting with a null model that included only "trial number" as a random explanatory variable. As our primary interest was the effect of penis handedness on male reproductive success, we added "treatment" to create the base model. The significance of this factor was tested by a likelihood ratio test (LRT) between the null

and base models. If a significant effect of "treatment" was detected, the following factors were sequentially added in a stepwise manner: recording position, male body size, female body size, male age and female age; goodness of fit was compared using a LRT. Our post hoc analysis revealed that male body size, female body size, male age and female age were not significantly different between the treatment groups (Table S1).

When a significant treatment effect was detected, the same analysis was applied for post hoc, pairwise multiple comparisons between treatment groups. Significance thresholds in multiple comparisons were corrected using the FDR. The 95% confidence intervals of each effect were calculated using the "effect()" function implemented in the package "effects."

3 | RESULTS

3.1 | Population study

From northern Japan to the southern Peninsular Malaysia (2.23–43.3° north), *L. riparia* males consistently showed a strong right bias in their penis resting state (88.7%; Figure 2). For Japanese populations, Kamimura (2006) reported that damaged penises and/or virgae occurred at a significantly higher rate in L-ready males (6 of 18, 33%) than in R-ready males (3 of 197, 1.5%), suggesting that the left penis is used as an extra organ when genital problems occur in the right penis. In the present study, a damaged right penis lobe was detected in 3 of 13 (23%) field-caught, L-ready males from the Malaysian populations (one each from the CH, ML and PN populations; Figure 1c). Similar damage was not detected in 70 field-caught, R-ready males from those populations (Fisher's exact probably test; $p = 0.0031$). Excluding the males with genital damage, only 30 of 350 (8.6%; range, 2.3% [IS] to 20.6% [CH]) were L-ready.

3.2 | Reproductive success of penis-ablated males

Overall, pairs mated up to seven times (mean ± SD, 1.65 ± 1.43), resulting in a total copulation duration of 30.3 ± 21.1 min (range, 0–59 min) during the 1-hr observation period. In all, 26 pairs (3 *Ctl*, 7 *RH* and 16 *LH*) did not copulate despite at least one copulation attempt from the males. One female that copulated for 13 min had detectable sperm in the spermatheca, whereas two females that mated for 27 and 45 min each were apparently not inseminated. These results suggest considerable variation in the timing of sperm transfer after attachment of the male and female genitalia.

The copulation success (occurrence of at least one copulation during the 1-hr observation period) and insemination success (transfer of a detectable amount of sperm) significantly varied among the treatments (Table 2; GLMM; $\chi_1^2 = 27.3$ and 50.4, respectively, both $p < 0.00001$). The addition of other explanatory variables (body size and age of males and females, and position in the video recording) did not significantly improve the model fit, except for a marginally significant effect of male body size on copulation and insemination success (Table 2). In pairs with at least one copulation (13 min or longer), the total copulation duration was significantly different among the treatment groups, whereas the latency to the first copulation was not (Table 2).

A post hoc analysis revealed that *RH* and *Ctl* males had a significantly higher copulation success than did *LH* males (Figure 3a), yielding odds ratios (ORs) of 3.50 and 9.41, respectively. Significantly more *Ctl* males than *LH* males inseminated the female (OR = 6.82), whereas the difference between *RH* and *LH* males was marginal ($p = 0.059$: OR = 2.94) after correction for multiple comparisons (Figure 3b). In contrast, the total copulation duration was significantly shorter in *RH* males than in *Ctl* or *LH* males (Figure 3c), regardless of their highest copulation frequency among the treatment groups (Table S1).

TABLE 2 Mixed model analyses to assess the effects of fixed factors on four measures of the reproductive success of *Labidura riparia* males

	Copulation success	Insemination success	Total copulation duration (min)[a]	Latency to the first copulation (min) [a]
Null model (~ Trial)				
Base model (~ Trial + Treatment)	0.00084 ($\chi_2^2 = 14.16$)	0.0022 ($\chi_2^2 = 12.27$)	0.0015 ($\chi_2^2 = 13.03$)	0.24 ($\chi_2^2 = 2.85$)
Additional explanatory variables				
+ Position	0.10 ($\chi_1^2 = 2.65$)	0.18 ($\chi_1^2 = 1.76$)	0.20 ($\chi_1^2 = 1.64$)	NA[b]
+ Male body size	0.058 ($\chi_1^2 = 3.60$)	0.052 ($\chi_1^2 = 3.76$)	0.39 ($\chi_1^2 = 0.74$)	NA[b]
+ Female body size	0.29 ($\chi_1^2 = 1.12$)	0.23 ($\chi_1^2 = 1.47$)	0.29 ($\chi_1^2 = 1.11$)	NA[b]
+ Male age	0.52 ($\chi_1^2 = 0.41$)	0.55 ($\chi_1^2 = 0.36$)	0.51 ($\chi_1^2 = 0.44$)	NA[b]
+ Female age	0.19 ($\chi_1^2 = 1.69$)	0.42 ($\chi_1^2 = 0.66$)	0.56 ($\chi_1^2 = 0.35$)	NA[b]

Note: p-values are given with χ^2 in parentheses.
[a]Only pairs with at least one copulation bout were analysed. [b]Not analysed since no significant effect of Treatment was detected.

FIGURE 3 Effects of artificial penis-ablation treatments (right penis ablated [*LH*], left penis ablated [*RH*] and neither penis ablated [*Ctl*]) of *Labidura riparia* on four measures of male reproductive success: (a) copulation success, (b) insemination success, (c) total duration of copulation and (d) latency to the first copulation. *p*-values shown above the bars are results after correction for multiple comparisons using FDR. Error bars indicate the 95% confidence intervals
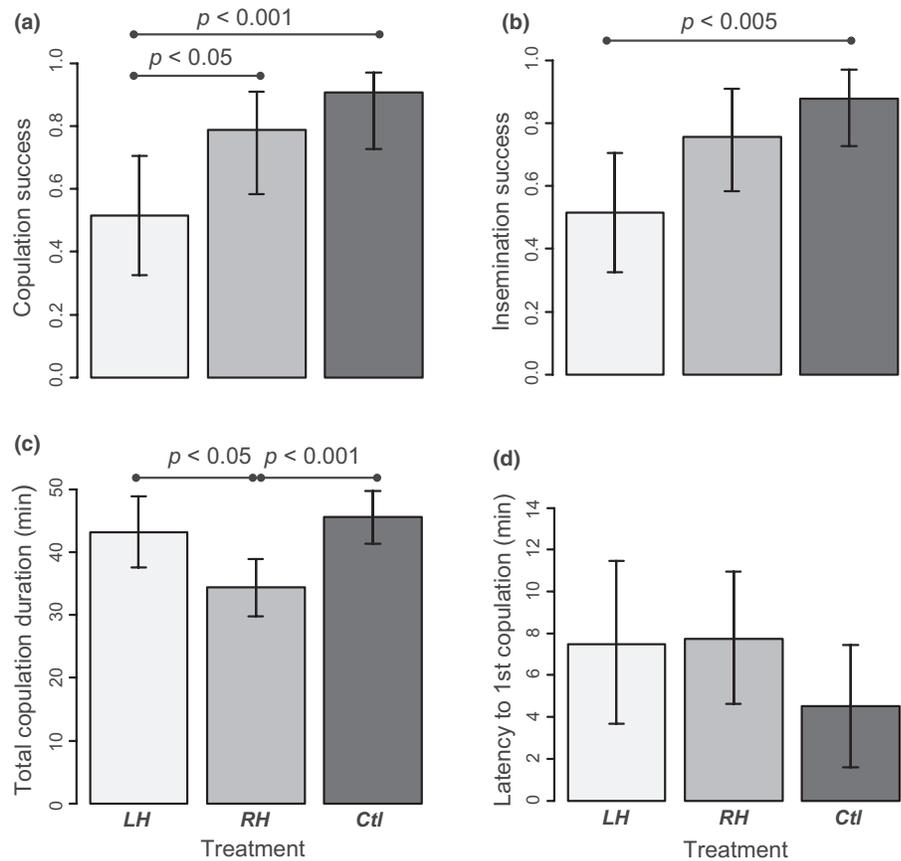
3.3 | Coupling of male and female genitalia

The direction of the spermathecal opening notably varied among the females examined, possibly due to its elastic nature (Figure 4a,b). However, the direction of coiling was always sinistral (as reported previously by Kamimura & Lee, 2014b), and the average direction was strongly biased to the right side of the female body (the mean angle and its 95% confidence interval were 58.4 ± 67.1° right from the anterior direction; Figure 4b). Given the morphology of the male intromittent organ (virgae), which curves slightly inward, we predicted that the opening direction of the spermatheca allow more smooth insertion of the right virga than the left one (Figure 4c vs. d).

We successfully examined four *RH*, two *LH* and three *Ctl* copulating pairs fixed at 5 min after copulation initiation. The other *RH* (*n* = 2), *LH* (*n* = 7) and *Ctl* (*n* = 2) pairs were separated before being transferred into the BABB solution. All of the *Ctl* males used their right penis for copulation. Although the differences could not be statistically tested because of the small sample sizes, the success rate of fixation was lower for the *LH* males (22.2%) than for the *RH* (66.7%) and *Ctl* males (60.0%), suggesting that the left virgae are inserted more shallowly or are released from the spermathecal opening more easily compared to the right ones. Although we could successfully fix only two pairs in which the male was *LH*, one male virga was notably kinked in the middle (Figure 4f), as predicted by its curvature and the direction of the spermathecal opening (Figure 4d). No such configuration was found in the other samples (*n* = 8; Figure 4e).

4 | DISCUSSION

4.1 | Advantage of right-handed males

To our knowledge, this study provides the first estimates of the benefits in major fitness components of biased use of laterally paired, morphologically symmetrical organs in an invertebrate species. The surgical manipulation, which mimicked natural penial damage, revealed that the right penis of *L. riparia* outperforms the left in copulation and insemination success, yielding OR of 3.50 and 2.94, respectively (Figure 3a,b; Table 3A). As we housed pairs individually, this difference in reproductive success could be larger in wild earwigs that may face pressure from male–male competition. In cases in which the corresponding right and left genital parts show differences in shape, surgical ablation of one side generally results in a much larger reduction in fitness components compared with ablation of the other side (Table 3B). For example, in the damselfly *Calopteryx cornelia*, males without the left aedeagal lateral process cannot remove rival sperm from the female spermathecae (Tsuchiya & Hayashi, 2014). In contrast, ablation of the right process, which is less developed than the left one, does not affect this measure of male reproductive success compared with unmanipulated controls (Tsuchiya & Hayashi, 2014). An exceptional case has been reported for the male genitalia of *Drosophila pachea*: ablation of either the right or left male genital lobe (epandrial lobes), in which the left lobe is much longer than the right, resulted
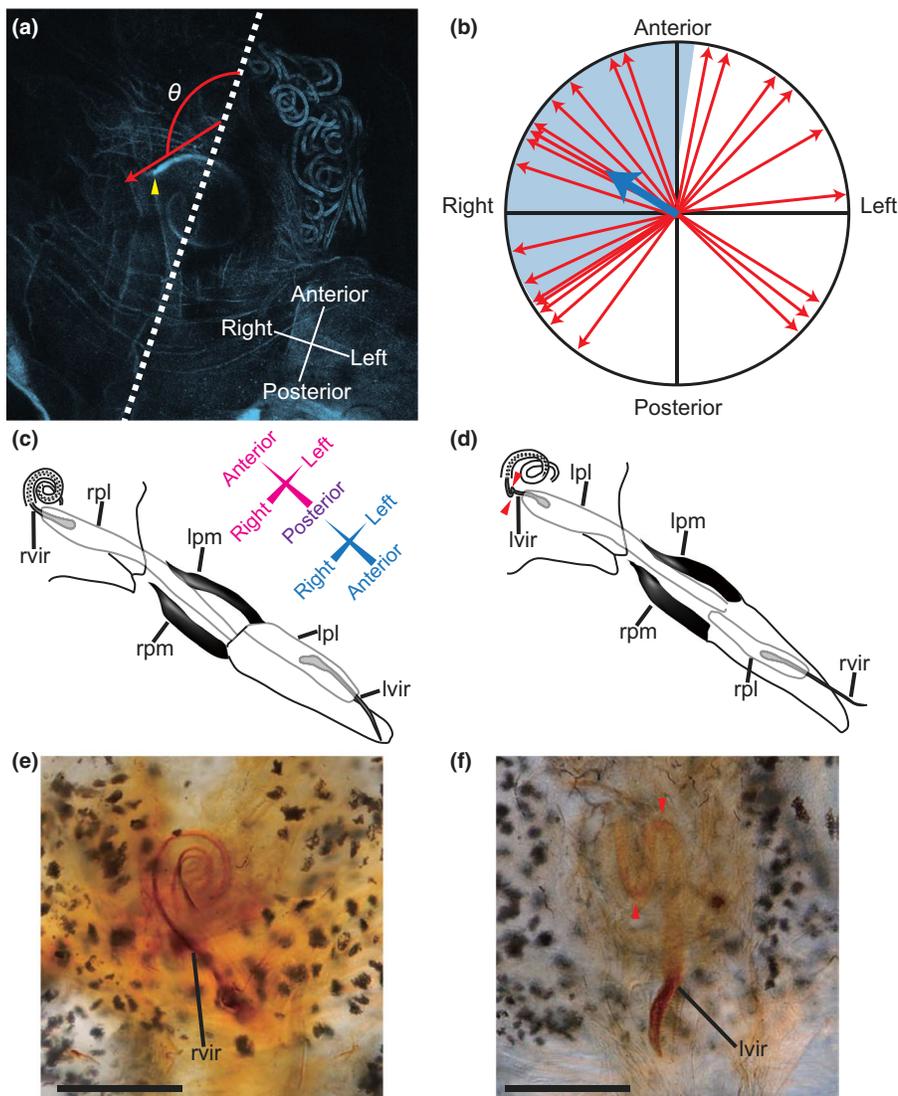
FIGURE 4 Direction of the spermathecal opening (a, b) and genital coupling (c–f) of *Labidura riparia*. (a) Laser scanning confocal micrograph of a spermatheca with its opening (yellow arrowhead), showing the measurement of the opening angle (θ) in relation to the opening direction (red arrow) and anterior–posterior axis (white dotted line). (b) Distribution of spermathecal opening angles (θ) in females. The mean angle (blue arrow) and its 95% confidence intervals (light blue sector) were calculated by the method shown in Zar (2009). (c, d) Expected coupling of the genitalia with (c) a right- and (d) left-handed male. (e, f) Examples of the observed couplings of the genitalia with (e) a right- and (f) left-handed male. The kinked parts in the virga are indicated by red arrowheads. The directions of the male (blue) and female (red) bodies are shown in (c). lpl, left penis lobe; lpm, left paramere; lvir, left virga; rpl, right penis lobe; rpm, right paramere; rvir, right virga. Scale bars: 250 µm

in abnormal male positions during mating with equal frequency (Rhebergen, Courtier-Orgogozo, Dumont, Schilthuizen, & Lang, 2016). However, the precise positions of each lobe and mating angle between the male and female bodies were different between the two treatments (Rhebergen et al., 2016), indicating a possible effect on male reproductive success.

We detected a similar trend for morphological asymmetries associated with shell-shape chirality of snails (OR: 8.47–107.1; Table 3C-a and 3C-b). Many species of terrestrial snails show dextral coiling in their shell morphology. Because male and female genital openings are on the right side of the body, rare sinistral mutants that possess genital openings on the opposite side experience lower success in penial insertion (Table 3C-a). Similarly, predators of snails, which possess specialized, laterally asymmetrical morphologies to handle dextral snails, experience significantly reduced success when attacking sinistral individuals (Table 3C-b). Thus, although the number of relevant studies was too small to conduct statistical tests, our comparisons indicate that relatively weak benefits result in the biased use of laterally paired organs compared to cases where morphological asymmetries are pronounced.

4.2 | Relationship with the evolution of asymmetrical morphology

A slight disadvantage of reversed handedness alone cannot explain the evolutionary retention of the less functional left penis. A reduction in penis number, from two to one, has independently occurred several times in Dermaptera phylogeny (as reviewed in Kamimura (2014)), as has the evolution of handedness from the ancestors that likely used both penises equally (Kamimura, 2004a; Kamimura & Lee, 2014a). The common ancestor of the superfamily Forficuloidea, which includes approximately 1,000 extant species in five families (Engel and Haas (2007) included only Spongiphoridae, Chelisochidae, Forficulidae and Arixeniidae, but recent phylogenetic studies (Klass, 2001; Jarvis et al., 2005; Kocarek et al., 2013; Naegle et al., 2016) indicate the inclusion of Hemimeridae in this clade), is hypothesized to have lost its left penis (Kamimura, 2006; but see Jarvis et al. (2005); Bilinski, Kocarek, Jankowska, Kisiel, & Tworzydlo (2014); Naegle et al. (2016) for the possible polyphyly of Forficuloidea: Spongiphoridae). Given the proposed sister relationships between *L. riparia* and Forficuloidea (see Introduction1), it is

TABLE 3 Odds ratios for the differential performances of the right and left organs revealed by controlled experimental studies

Study species	Fitness component	Odds ratio (95% confidence limit)	Source
Category A: Biased use of morphologically symmetrical genital organs			
Earwig (*Labidura riparia*)	Copulation success	3.50 (1.19–10.3)	This study
Earwig (*Labidura riparia*)	Insemination success	2.94 (1.03–8.39)	This study
Category B: Biased use of morphologically asymmetrical genital organs			
Damsel fly (*Calopteryx cornelia*)	Removal of rival sperm	∞	Tsuchiya and Hayashi (2014)
Praying mantis (*Tenodera aridifolia*)	Opening female genitalia	∞	Hashimoto, Suzuki, and Hayashi (2016)
Praying mantis (*Tenodera aridifolia*)	Spermatophore transfer	∞	Hashimoto et al. (2016)
Fruit fly (*Drosophila pachea*)	Establishing normal mating posture	3.67 (0.62–21.7)	Rhebergen et al. (2016)
Category C-a: Morphological asymmetry related to snail chirality: mating between same/different morphs			
Snail (*Bradybaena similaris*)	Copulation success	107.1 (31.8–360.6)	Asami, Cowie, and Ohbayashi (1998)
Snail (*Partula suturalis*)	Copulation success	8.47 (2.19–32.8)	Asami et al. (1998)
Category C-b: Morphological asymmetry related to snail chirality: predation on dextral/sinistral morphs			
Crab (*Eriphia smithii*)	Predation success	27.8[a]	Shigemiya (2003)
Water scavenger (*Hydrophilus acuminatus*)	Predation success/ preference	16.1[b]	Inoda, Hirata, and Kamimura (2003)
Snake (*Pareas iwasakii*)	Predation success	9.09[c]	Hoso, Asami, and Hori (2007)

Note: Odds ratios and 95% confidence intervals were calculated using the "oddsratio()" function in the "fmsb" package, except for the studies indicated by asterisks.

[a]From Table 3 of Shigemiya (2003). [b]Estimated based on the data presented in Figure 2 of Inoda et al. (2003). [c]Estimated based on the data presented in Figure 2c of Hoso et al. (2007).

likely that the lower reproductive success of left-handed males resulted in the evolutionary loss of left penises in a common ancestor of Forficuloidea.

However, the risk of accidental breakage of the right penis may evolutionarily maintain the left penis, which many males do not use at all during their lifetime, in *L. riparia* (Kamimura, 2006; this study). Breakage of a virga under natural conditions has also been reported in anisolabidid males (*Euborellia plebeja* and *Anisolabis maritima*), which have a pair of virgae longer than their bodies (Kamimura & Matsuo, 2001). Virgal breakage in these species is frequently accompanied by necrosis of a penis lobe, as observed in *L. riparia* (Kamimura & Matsuo, 2001; Kamimura, Tee, & Lee, 2016). *Marava arachidis* males (Spongiphoridae) possess a single but extremely elongated virga, which is inserted into the long tube-shaped spermatheca during insemination (Kamimura et al., 2016). The thin virga sometimes breaks during copulation, but unlike earwigs possessing an extra virga, genital damage is restricted to the virgal tip, without which males can still inseminate females (Kamimura et al., 2016).

These patterns of genital accidents suggest that a single penis lobe of the Forficuloidea is more robust than those of the earwigs with a spare penis. Comprehensive studies on the fragility of earwig penises are necessary to test this hypothesis.

4.3 | Genital coevolution and possible polymorphisms

There is ongoing debate on the major driving force underlying the evolution of population-level laterality and associated polymorphisms. Human handedness, which may date back ~1.8 million years to *Homo habilis* (Frayer et al., 2016), is considered to stem from cerebral lateralization, in that reversal of the functional differentiation between the right and left brain hemispheres is correlated with left-handedness (e.g. Annett, 2013; Harris, 1991; McManus, 2004). Cerebral lateralization is considered advantageous, as it may conserve limited neural resources and avoid confusion within neural networks (Rogers, Vallortiagara, & Andrew, 2013; Vallortigara, 2006;

Vallortigara & Rogers, 2005; Vallortigara et al., 2011). Although left-handed individuals are diagnosed with developmental disorders more often than right-handed individuals (Coren & Halpern, 1991; Hughes, Donner, & Wind, 2008; Llaurens, Raymond, & Faurie, 2009), approximately 8% of people today are left-handed, indicating that certain factors counter these potential fitness costs. Game theory models predict that frequency-dependent costs and benefits can result in an evolutionarily stable coexistence of right- and left-handed individuals. Such frequency-dependent costs and benefits may arise from antagonistic and synergistic interactions among members of a species (Abrams & Panaggio, 2012; Ghirlanda, Frasnelli, & Vallortigara, 2009) or predator–prey interactions (Ghirlanda & Vallortigara, 2004). In the case of humans, the less common left-handers likely have an advantage in hand-to-hand combat (Faurie & Raymond, 2004; Raymond, Pontier, Dufour, & Møller, 1996). Evidence from invertebrates also supports this hypothesis (Niven & Frasnelli, 2018). Social hymenopterans (e.g. honeybees) show lateralized antennal use in learning processes (Letzkus, Boeddeker, Wood, Zhang, & Srinivasan, 2007; Letzkus et al., 2006), whereas no similar behaviour is observed among nonsocial hymenopterans (e.g. solitary mason bee) (Anfora, Frasnelli, Maccagnani, Rogers, & Vallortigara, 2010). However, both eusocial honeybees (Rogers, Rigosi, Frasnelli, & Vallortigara, 2013) and solitary mason bees (Rogers, Frasnelli, & Versace, 2016) exhibit population-level laterality of the antennal use in social interactions, indicating the importance of social pressure for the evolution of laterality (Frasnelli & Vallortigara, 2018).

Male–female interactions in courtship and mating represent another category of population-level laterality, with increasing reports of examples from insects (reviewed in Niven & Frasnelli (2018)). For example, observational studies demonstrated that the males of some beetle species frequently approach females from a particular side and experience a higher mating success than those that approach from the opposite side (Benelli, Romano, Kavallieratos, et al., 2017; Benelli, Romano, Stefanini, et al., 2017). Frequency-dependent selection can also maintain polymorphisms associated with the male behaviour of this category. For example, if females resist coercive mating attempt by males, females likely spend more time vigilant of the side from which more males try to mount them. Thus, males approaching them from another side will have an advantage. However, to our knowledge, no study has demonstrated this type of frequency-dependent selection for insect courtship behaviour and genital interactions (but see Holwell, Kazakova, Evans, O'Hanlon, & Barry (2015), for a possible case).

Our observations of genital couplings strongly suggest that the spirally coiling morphology of the female spermatheca allowed for smoother and deeper insertion of the right virga than of the left one. To our knowledge, striking spiral of the spermathecal opening region is unique to the genus *Labidura* among earwigs (Hudson, 1973; Kamimura & Lee, 2014b). Despite the advantage of right-handed penises demonstrated in this study, approximately 8.6% of *L. riparia* males showed an L-ready state without any signs of damage to the right penis. This result resembles, at least superficially, human handedness. However, we failed to detect any

corresponding polymorphisms in female morphology: the opening region of the spermatheca consistently shows sinistral coiling in Japanese and Malaysian females (Kamimura & Lee, 2014b). Furthermore, even if a small fraction of females possess a dextrally coiled spermatheca, all males should be R-ready if they cannot determine female type before mating. Thus, the evolutionary origin of indigenously left-handed male *L. riparia* is unknown at present. A quantitative genetic study of *E. plebeja* males, which use their right and left penises equally (Kamimura & Matsuo, 2001), showed no additive genetic basis for their penis handedness (Kamimura & Iwase, 2010). Given the relatively large across-population variation (2.3%–20.6%) in the proportion of L-ready males without genital damage, future studies should explore whether inborn left-handers of *L. riparia* reflect a phenotypic variation or a genetically controlled one.

4.4 | Significance of female spermathecal morphology

Despite having the highest copulation frequency (Table S1), the total copulation duration was shorter in *RH* males compared with *LH* and *Ctl* males, the latter of which likely included some males that used the left penis for insemination. This result is also consistent with the view that the spermathecal morphology allows for a smoother insertion of the right virga, leading us to wonder why female *L riparia* have a conspicuously coiled and elongated spermatheca.

Female Muscovy ducks (*Cairina moschata*) also have extensively coiled vaginas, into which males insert a corkscrew-like penis, coiled in the opposite direction, during copulation. Experiments using 3D glass models demonstrated that the vaginal morphology makes insertion of the oppositely coiled male penis difficult. This suggests that females may resist coercive mating, which is common in this group of animals (Brennan, Clark, & Prum, 2010). In *L. riparia* and many other earwigs, female quiescence is usually necessary to establish genital coupling (Kamimura, 2014). In addition, since most males are right-handed, the virga usually used for copulation matches the direction of the spermathecal coiling. Unlike in waterfowls, the spermathecal coiling is not likely to function as an adaptation to counter coercive copulation attempts.

In insects, extensively coiled spermathecae with reverse turns have been reported in leaf beetles of the subfamily Cassidinae. Theoretical studies have demonstrated that reverse turns make it difficult for males to propel their elongated genitalia (flagellum) into the spermatheca (Filippov, Kovalev, Matsumura, & Gorb, 2015; Filippov, Matsumura, Kovalev, & Gorb, 2016). Interestingly, in the Panamanian species *Chelymorpha alternans*, females ejected male seminal fluid more often when male flagella were experimentally shortened (Rodriguez, Windsor, & Eberhard, 2004), indicating cryptic female choice on male ability to deeply insert the flagellum. Brousse-Gaury (1985) reported that two groups of sensilla, which likely function as mechanoreceptors, are asymmetrically distributed around the spermathecal opening of *L. riparia*: a group of sensilla anterior to the spermathecal opening on the right side and another

group in the left side, more posteriorly to the right group. This observation suggests the possibility that female *L. riparia* also actively choose males based on their penis handedness. However, as shown in a previous (Kamimura, 2006) and the present study, a majority of the left-handed males have a damaged right penis. As with many other earwig species (Briceño & Eberhard, 1995), males of *L. riparia* likely use sexually dimorphic, exaggerated forceps for acquiring mates, which implies that accidental genital damage is not negatively correlated with male genetic quality. Alternatively, the asymmetrically arranged sensilla might function to sense proper genital coupling with a right-side penis. In *E. plebeja*, the spermatheca is also elongated to twice the body length (Kamimura, 2000). This structure is considered an adaptation to selectively accumulate sperm from large-sized males, which are capable of repeating partial displacement of the stored sperm using their elongated virgae (Kamimura, 2015). Although *L. riparia* females also mate multiple times, the sperm storage dynamics are not yet known in this species. Further studies on their reproductive biology are warranted to evaluate the ultimate evolutionary basis of right-handedness and the possible loss of left penises in this group of insects.

CONFLICT OF INTEREST

There are no conflicts of interests to declare.

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