npg

ORIGINAL ARTICLE

The genetic basis of interspecific differences in genital morphology of closely related carabid beetles

M Sasabe, Y Takami and T Sota

Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto, Japan

Marked diversification of genital morphology is common in internally fertilizing animals. Although sexual selection may be the primary process controlling genital evolution, factors promoting genital evolution are controversial, and the genetic background of genital morphology is poorly understood. We analyzed the genetic basis of species-specific genital morphologies in carabid beetles of the subgenus *Ohomopterus* (genus *Carabus*, Carabidae) using two parapatric species with hybrid zones. Biometric analyses on experimental F_1 and backcross populations revealed that inheritance of genital morphology is polygenic. Applying Lande's modification of the Castle–Wright estimator to population means and variances to estimate the minimum number of genes involved, we found that a relatively small number of loci is responsible for species differences in genital morphology. In addition, joint-scaling tests indicated that the additive genetic effect accounts for most interspecific differences in genital traits, but dominance and epistatic genetic effects also play roles. Overall, the genetic basis of male and female genitalia is fairly simple, enabling these traits to respond quickly to selection pressures and to diverge rapidly. Our results provide insight into the diversification of genital morphology in carabid beetles, and will hopefully stimulate further studies on the genetic basis of genitalia, such as mapping of quantitative trait loci affecting species-specific genital morphology.
Heredity (2007) **98**, 385–391; doi:10.1038/sj.hdy.6800952; published online 28 February 2007

Introduction

Marked diversification of genital morphology is often observed in animals with internal fertilization, especially in arthropods (Eberhard, 1985). Although the process leading to such diversification is a matter of controversy in evolutionary biology, the most plausible driving force of genital evolution is sexual selection, in the form of sperm competition, cryptic female choice and sexual conflict (Eberhard, 1985; Hosken and Stockley, 2004), and several lines of empirical evidence have been presented (eg, Arnqvist, 1998; Arnqvist and Danielsson, 1999; Danielsson and Askenmo, 1999; House and Simmons, 2003; Wenninger and Averill, 2006). However, the genetic basis for the variation in genital morphologies amongst related animals has not been well explored, although it is essential for understanding rapid diversification of reproductive traits under sexual selection or other evolutionary processes. Only two studies have elucidated the genetic basis of male genital morphology in relation to its function: a heritability estimation in a water strider (Arnqvist, 1989) and an analysis of genetic variance and covariance structure in a dung beetle

(House and Simmons, 2005). Two quantitative trait locus (QTL) analyses have been conducted examining genital morphology in *Drosophila* flies (Liu *et al.*, 1996; Zeng *et al.*, 2000), but neither focussed on the functional morphology of genitalia (cf., Jagadeeshan and Singh, 2006).

In this study, we explore the genetic basis of the interspecific morphological differences in functional parts of genitalia in the carabid beetles of the subgenus *Ohomopterus* (genus *Carabus*, Carabidae). *Ohomopterus* is a species-rich group endemic to Japan and exhibits extreme diversity in genital morphology (Ishikawa, 1991; Takami and Sota, in press). The genitalia of *Ohomopterus* show an elaborate lock-and-key system with characteristic male and female genital parts, that is, the copulatory piece, the sclerotized portion of the membranous part (endophallus) of the male genitalia and its receptacle, the vaginal appendix, a pocket attached to the female vagina. In copulation, a copulatory piece inserted into a vaginal appendix works as an anchor to secure coupling of genitalia (Ishikawa, 1987; Takami, 2003). The copulatory piece and vaginal appendix match in size and shape within species or subspecies, and are thought to work as an agent of mechanical isolation (Ishikawa, 1987).

The shape of the copulatory piece is highly variable amongst species of *Ohomopterus* and can be hooklike, triangular or pentagonal. The triangular copulatory piece is small and represents an ancestral type, whereas the pentagonal and hooklike types with generally enlarged sizes are derived (Sota and Vogler, 2003). In a derived lineage with pentagonal to hooklike copulatory pieces,

Correspondence: *M Sasabe, Department of Zoology, Graduate School of Science, Kyoto University, Kitashirakawa-oiwake, Sakyo, Kyoto 606-8502, Japan.*
E-mail: msasabe@terra.zool.kyoto-u.ac.jp

closely related species with different genital morphologies form hybrid zones at their boundaries. At the hybrid zone, the interspecific differences in genital morphology incur a large cost for interspecific mating because of the mismatch of male and female genital morphologies. This has been demonstrated experimentally in *Ohomopterus iwawakianus* and *O. maiyasanus*, parapatric species with very different genital morphologies (Sota and Kubota, 1998). In this pair of species, however, the mechanical isolation is not complete, and heterospecific pairs often produced viable F_1 offspring that were fertile when backcrossed or intercrossed, indicating that post-zygotic isolation has not been accomplished between these species. Therefore, these two species were chosen to examine the genes responsible for establishing species differences in genitalia using interspecific hybridization.

In this paper, we analyze the genetic background of genitalia in the carabid beetles *Ohomopterus* by quantitative genetic analysis with the Castle–Wright estimator (Lande, 1981) and the joint-scaling test (Mather and Jinks, 1982; Lynch and Walsh, 1998) based on interspecific hybridization and backcrossing experiments. Our results provide insights into the genetic architecture underlying the diversification of genitalia in *Ohomopterus*.

Materials and methods

Study organisms and sampling
Two species of carabid ground beetles, *O. iwawakianus* and *O. maiyasanus* (hereafter *iwawakianus* and *maiyasanus*, respectively) were used in this study. These species are similar in external morphology, but their genitalia are very different, especially in the copulatory piece and vaginal appendix, the functional parts used in the coupling of genitalia (Figure 1; see also Sota and Kubota,

1998). The adult *iwawakianus* beetles used in the experiments were collected at Kameyama in the Mie Prefecture, and those of *maiyasanus* were collected at Mt. Uryuzan in Kyoto, Kohoku in Shiga, and Suzuka in Mie Prefecture, Japan. Overwintering, pre-reproductive beetles were collected from the soil in winter and early spring, and kept in an incubator under a 12L:12D light regime and at $4 \pm 1°C$ until experiments were started. Females and males were kept in separate plastic containers (3–5 individuals per container, 9 cm diameter, 9 cm height), and were fed minced beef and apple slices every 2–3 days. In spring, incubator conditions were gradually shifted to 16L:8D at $20 \pm 1°C$, 2 weeks prior to the hybridization experiments.

Interspecific hybridization and backcrossing
In 1996 and 2003, interspecific hybridization experiments were carried out by crossing reciprocal pairs of *iwawakianus* and *maiyasanus* to obtain F_1 populations. We hereby define P_i as *iwawakianus*, P_m as *maiyasanus*, and BC_i and BC_m as backcrosses to *iwawakianus* and *maiyasanus*, respectively. The initial crossing conducted in 1996 included 2 pairs of *maiyasanus* female × *iwawakianus* male, and the more expanded crossing in 2003 used a total of 76 reciprocal pairs (43 pairs of *maiyasanus* female × *iwawakianus* male; 32 pairs of *iwawakianus* female × *maiyasanus* male). The F_1 progeny were then backcrossed to the parental species in 1997 and 2004 to yield BC_i (F_1 × *iwawakianus*) or BC_m (F_1 × *maiyasanus*) individuals. In 1997, 8 pairs of backcrosses were set (4 pairs of F_1 × *iwawakianus* and 4 pairs of F_1 × *maiyasanus*), and in 2003, 82 pairs were set (41 pairs of F_1 × *iwawakianus* and 41 pairs of F_1 × *maiyasanus*). We did not use within-family crossing in this study, and a female was allowed to mate with a single male.
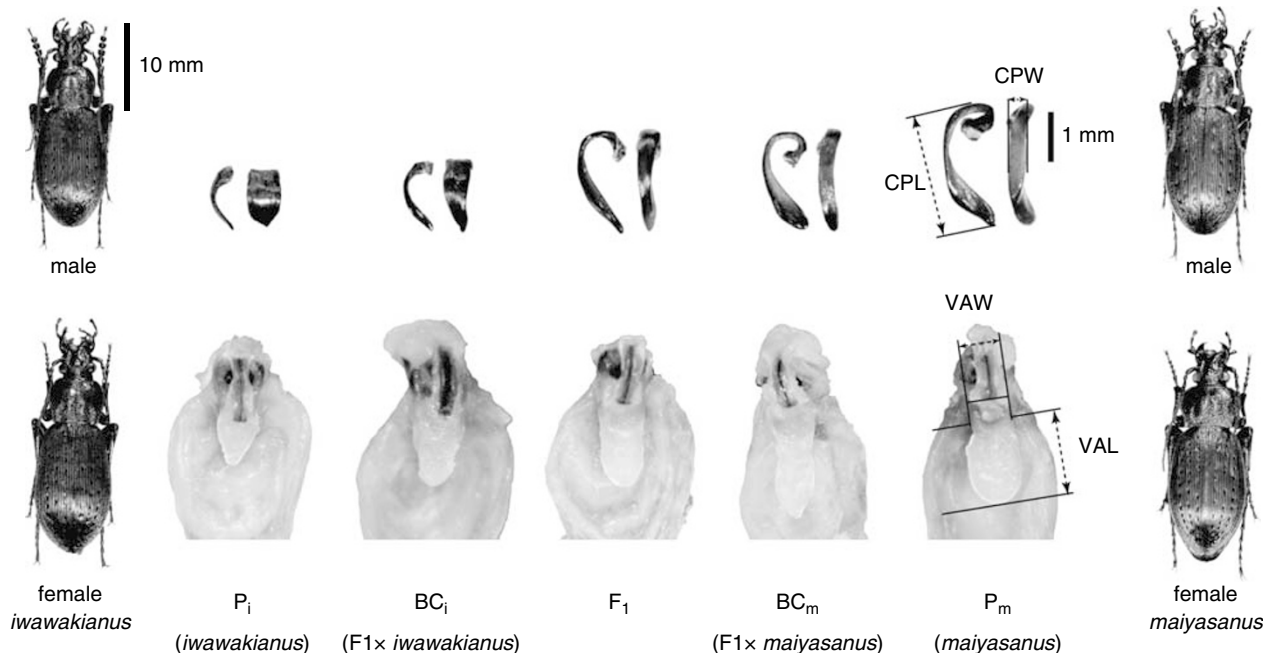
Figure 1 Male and female genital morphology of *O. iwawakianus* (left), *O. maiyasanus* (right) and their hybrids. External morphologies of parents and measurements of genital traits are also presented. CPL, CPW, VAL and VAW refer to copulatory piece length, copulatory piece width, vaginal appendix length and vaginal appendix width, respectively. Lateral and ventral views of copulatory piece, and ventral view of female genitalia are shown.

Hybrid larvae were reared individually in plastic cups (5.5 cm diameter × 3 cm height) from hatching to the third (final) instar and fed on live earthworms. At the late third instar, each individual was transferred to a deeper plastic cup (5 cm diameter × 7 cm height) filled with sieved soil (approximately 5 cm deep) to allow for pupation. After eclosion, emerged adults were kept individually for about 2 months and fed minced beef and sliced apples until skeletal maturation.

Morphological analysis of genitalia

The male aedeagus, an intromittent organ, was dissected, and the endophallus (membranous extention of the aedeagus) was everted by injecting toothpaste from the base of the aedeagus using a syringe. The copulatory piece on the endophallus was carefully removed with fine forceps for subsequent measurement. Images of the copulatory piece were digitized using a Keyence VHX-100 (×40 magnification) microscope, and the length and width of copulatory pieces (hereafter CPL and CPW, respectively) were measured on the digitized images as shown in Figure 1. For females, the reproductive organ was surgically removed, as were the muscles around the vagina. Vaginal appendix length and width (VAL and VAW, respectively) in the fully extended form were measured on the Keyence VHX-100 image (Figure 1). Pronotum width was also measured to the nearest 0.01 mm for each individual, as a reference to body size, using a digital calliper. For each population mean of traits, departures from normality were tested by Shapiro–Wilk test statistics.

Biometric analyses of genital traits

To estimate the number of effective factors involved in interspecific differences in genital phenotype, we applied Lande's (1981) modification of the Castle–Wright estimator to each population (P_i/P_m, F_1, BC_i/BC_m). This estimator is based on a biometric method using mean phenotypic value and variance to estimate effective (minimum) number of genetic factors. The estimator depends on several assumptions: loci involved must be additive and unlinked, and contribute equally to phenotypic differences between lines, and loci increasing a trait value are fixed in one parental population and those decreasing a trait value are fixed in another parent. Violations of one or more assumptions tend to result in underestimates of the actual number of loci.

Zeng (1992) proposed a modification of the Castle–Wright estimator to include additional parameters, i.e., variation in the allelic effect (C_α) and recombination rate, and this might be worth applying to our data. Because there is no information on the distribution of allelic effects for carabid beetles, we used the half-normal distribution of the allelic effect (where $C_\alpha = 0.57$) and the constant effect model ($C_\alpha = 0$). The recombination rate of genes was estimated as $c = 0.464$ using haploid chromosome number ($n = 14$; Serrano and Galián, 1998; T Sota, unpublished) and the formula described in Lynch and Walsh (1998).

To check for the assumptions of the Castle–Wright estimator, we used a joint-scaling test based on an additive-dominance model and sequential parameter model fitting (Mather and Jinks, 1982; described in detail in Lynch and Walsh, 1998) to assess composite genetic effects (i.e., additive, dominant, or epistatic) underlying the morphological differences in genitalia. The joint-scaling test was carried out by weighing means using the reciprocals of the variances of the population. The adequacy of the best-fit model was evaluated using a chi-square test with the degrees of freedom determined as the number of populations minus the number of parameters in the model. A likelihood-ratio test statistic was used to determine if a significant proportion of variation was explained by the dominance parameter. The percentage of variation explained by the most significant parameter was used to assess the contribution of each genetic parameter in relation to the percent variation explained by the best-fit model (estimated as coefficient of determination, R^2). In case the simple additive-dominance model did not adequately explain species differences, we concluded that other higher-order genetic interactions, such as epistatic effects, were involved, and the percentage residual was considered to be the contribution of epistatic effects. Further estimation of epistasis was not valid by means of the joint-scaling test because not enough population means were available (i.e., no F_2) in our current experimental design.

Results

Hybridization experiments and genital morphology of hybrids

Interspecific hybridization experiments resulted in 235 F_1 individuals (113 females, 122 males) from 46 pairs (33 from *maiyasanus* female × *iwawakianus* male, 13 from *iwawakianus* female × *maiyasanus* male). Of these, 185 were obtained from *maiyasanus* female × *iwawakianus* male (8 from 1996 cross) and 50 were from *iwawakianus* female × *maiyasanus* male (none from 1996 cross). Although 82 pairs of backcross experiments were used, we obtained unexpectedly small numbers of backcross individuals: 21 BC_i progeny from 10 pairs of F_1 × *iwawakianus*, and 47 BC_m progeny from 11 pairs of F_1 × *maiyasanus*. Details of interspecific cross are provided in Table 1.

Male copulatory pieces of *maiyasanus* and *iwawakianus* were clearly distinguishable in CPL and CPW (Figure 2). Genital forms of F_1 individuals were approximately the intermediate of parental forms, although the mean phenotypic value for CPW was larger than the midparent value. Morphology of the backcross copulatory pieces was the intermediate of F_1 and the parental species.

Although female vaginal appendices were membranous and hence of flexible (but not elastic) structure, no overlap in VAL was observed between parental species (Figure 2). However, VAW values overlapped and were not distinguishable between species, and unlike males, the VAWs of *maiyasanus* were much wider than those of *iwawakianus*. The overall trend in VAL was similar to that of males (Figure 2); VAL values of F_1 were intermediate but slightly longer than the midpoint, and backcross daughters were more similar to parent species. Figure 2 indicates that genital traits are inherited in a quantitative genetic manner and are polygenic. Means and variances for VAW were not used in the subsequent analysis because no significant differences were detected amongst experimental populations.

Table 1 Sample sizes of experimental populations by cross type and year of experiment

Male population[a]	Cross type[a,b] (female × male)	Year		Total	Female population[a]	Cross type[a,b] (female × male)	Year		Total
		1996	2003				1996	2003	
P_i		1	32	33	P_i		1	20	21
P_m		0	22	22	P_m		3	24	27
F_1	m × i	2	94	96	F_1	m × i	6	83	89
	i × m	0	26	26		i × m	0	24	24
	Total	2	120	122		Total	6	107	113
BC_i	F_1 (m × i) × i	3	4	7	BC_i	F_1 (m × i) × i	3	3	6
	F_1 (i × m) × i	0	0	0		F_1 (i × m) × i	0	0	0
	i × F_1 (m × i)	3	0	3		i × F_1 (m × i)	2	3	5
	i × F_1 (i × m)	0	0	0		i × F_1 (i × m)	0	0	0
	Total	6	4	10		Total	5	6	11
BC_m	F_1 (m × i) × m	7	0	7	BC_m	F_1 (m × i) × m	0	0	0
	F_1 (i × m) × m	0	0	0		F_1 (i × m) × m	0	7	7
	m × F_1 (m × i)	5	2	7		m × F_1 (m × i)	4	6	10
	m × F_1 (i × m)	0	0	0		m × F_1 (i × m)	13	3	16
	Total	12	2	14		Total	17	16	33

[a]i, O. iwawakianus; m, O. maiyasanus.
[b]For example F_1 (m × i) × i represents backcross of F_1 female (from maiyasanus female × iwawakianus male) × iwawakianus male.

Variance components and the effective number of genetic factors

The distributions of values of genital dimensions did not depart from normality (Shapiro–Wilk test; $P > 0.05$), hence raw data were used in the following analyses. In addition, we did not normalise trait values using a size index (pronotal width here) because no significant correlations were observed between these values. There were no significant differences in the population means between data sets from 1996 and 2003. However, there was a small but significant difference between the directions of the cross in both traits of F_1 males (ANOVA; $F_{1,121} = 19.2$, $P < 0.0001$ for CPL; $F_{1,121} = 28.2$, $P < 0.0001$ for CPW; the cross effect explained 13.8 and 19.0% of the variance in CPL and CPW, respectively); the F_1 from the maiyasanus female had a larger CPL, but a smaller CPW (mean ± s.d. [mm]: maiyasanus vs iwawakianus 1.89 ± 0.11 vs 1.77 ± 0.14 for CPL, 0.48 ± 0.04 vs 0.43 ± 0.05 for CPW).

Genetic parameter estimates are described in Table 2. For CPL, chi-square values were not significant, indicating that the additive-dominance model adequately explained the data. In contrast, the additive-dominance model was inadequate to explain the interspecies variation for CPW and VAL, implying the contribution of higher-order interactions between loci, i.e., epistatic effects. Genetic parameter estimates listed in Table 1 were statistically significant in all cases.

The simple additive model explained 99.2% of variation in CPL, 89.7% in CPW, and 89.9% in VAL. For CPW, the best-fitting model included dominance and epistatic effects (9.3 and 1.0% of variation explained, respectively; Table 2). Significant dominance and epistatic effects accounted for the variation in VAL (6.8 and 3.3% of variation explained, respectively).

Because the assumption of additivity was met, we applied the Castle–Wright estimator. The estimated number of factors involved in interspecific differences in genitalia (± s.e.) was 4.4 ± 2.3 for CPL, 10.4 ± 9.1 for CPW, and 1.8 ± 1.4 for VAL. A trial application of Zeng's correction of the Castle-Wright estimator gave values of 6.0 ± 4.9 and 8.8 ± 7.6 for CPL when the allelic effects were assumed to be $C_\alpha = 0$ and $C_\alpha = 0.57$, respectively. For CPW, both allelic effect models yielded unreliable results with inflated standard errors overwhelming the estimated number (46.9 ± 198.0 for $C_\alpha = 0$; 73.1 ± 310.8 for $C_\alpha = 0.57$). The value for VAL was 1.9 ± 1.7 with an allelic effect of $C_\alpha = 0$, but again the standard error exceeded the estimated number when $C_\alpha = 0.57$ was applied (2.4 ± 2.7).

Discussion

The Castle–Wright estimator has been used to evaluate the genetic background of various quantitative characters relevant to adaptation and speciation in a variety of animals, including insects (eg, Shaw, 1996; Hatfield, 1997; Wijngaarden and Brakefield, 2000; Albertson et al., 2003; Huttunen and Aspi, 2003; Saldamando et al., 2005). Several studies on the utility of the Castle–Wright estimator have suggested that the difference between the actual number of loci (confirmed in a QTL mapping study) and the number estimated with the Castle–Wright estimator is small if assumptions are met (Otto and Jones, 2000; Westerbergh and Doebley, 2002). When additivity is violated, however, this estimator gives biased estimates (Zeng et al., 1990). Although incomplete dominance likely affected CPW and VAL, the genital traits studied here were inherited predominantly in an additive fashion, as additive effects explained most of the phenotypic variation in CPL (>99%), CPW (90%), and VAL (90%). Thus, we concluded that our estimations were fairly reasonable.

Zeng (1992) modification of the Castle–Wright estimator, which incorporated a variation in allelic effect and recombination rate, resulted in larger estimates of effective number of loci, as in Saldamando et al. (2005), but standard errors for CPW and VAL (at $C_\alpha = 0.57$) exceeded over estimated numbers. Applying recombination index and genetic effect of other organisms could cause questionable output. Thus, as stated in Lynch and
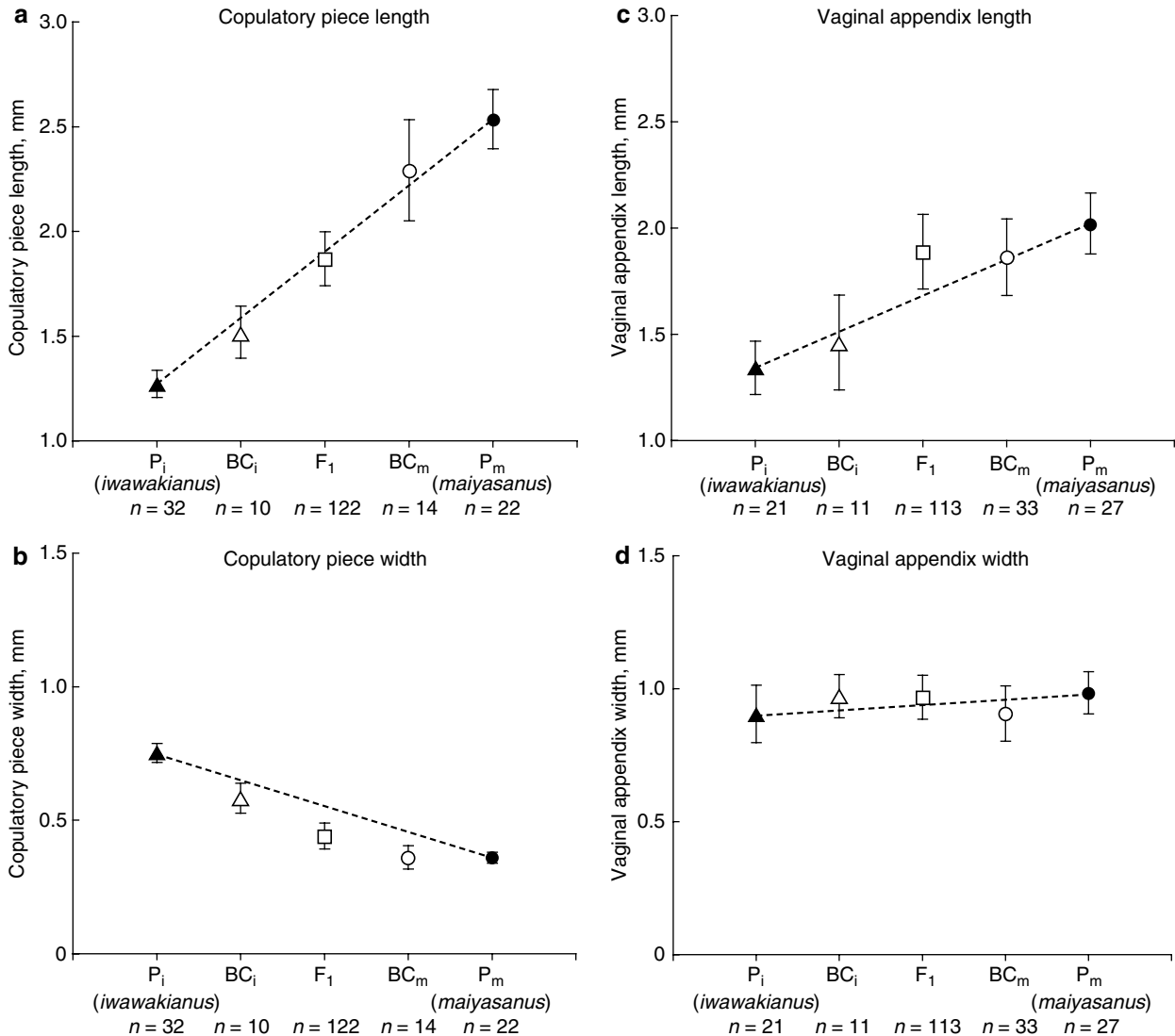
Figure 2 Means and standard deviations of genital morphology for two parental species and their hybrids. Dotted lines connect the means for parental species, providing a reference for deviations from a simple additive model, which predicts hybrid means locate on the lines.

Table 2 Genetic parameters estimated by additive-dominance model in joint-scaling test

Trait	(a) Parameters estimated by additive+dominance model					(b) % Explained by each parameter		
	$m\ (\pm s.e.)$	$[a]\ (\pm s.e.)$	$[d]\ (\pm s.e.)$	$\chi^2\ (df)$	P	$[a]$	$[d]$	% residual
CPL	1.908 ± 0.016	0.637 ± 0.016	-0.039 ± 0.020	3.585 (2)	0.1665	99.2	0.05	N/A
CPW	0.554 ± 0.004	-0.197 ± 0.004	-0.116 ± 0.006	11.184 (2)	0.0037	89.7	9.3	1.0
VAL	1.665 ± 0.019	0.329 ± 0.018	0.211 ± 0.025	8.401 (2)	0.0150	89.9	6.8	3.3

(a) m, midparent value; [a], additive effects; [d], dominance effects. Percentage of total variance explained by genetic models.
(b) N/A indicates that simple additive+dominance model was adequately fitted to population means.

Walsh (1998), this type of estimation should be treated with caution using very large sample sizes.

Although the Castle–Wright estimator ignores sex-linked genes, the male traits of the F_1 population differed significantly between reciprocal crosses, implying an effect of the sex chromosomes (note that *Ohomopterus* has an XY system) or maternal effect. The differences in the trait values between reciprocal crosses were fairly small compared with the large differences between parental species, and thus may not a have large effect on our estimate. Because the pattern of deviation in the F_1 male traits corresponded to that in the parental species (i.e., a longer, but narrower copulatory piece for *maiyasanus*), this provides a clue for a future study of

the genes causing the interspecific difference in genital morphology.

Our results revealed that relatively few loci are involved in genital morphology variation: 4.4 and 10.4 for the male traits and 1.8 for the female trait. A previous quantitative trait locus (QTL) mapping study using *Drosophila* species (Zeng *et al.*, 2000) revealed that at least 19 loci were involved in the species differences of male genitalia. The estimated numbers of loci in carabid beetles were much smaller, implying that the genetic basis enables the genital traits to respond to selection quickly. In particular, it was estimated that very few loci governed VAL, indicating that this trait is able to respond to selection more quickly, so that counter-adaptation against exaggeration in male characters could be attained to avoid physical damage to the vagina during copulation or to increase the ability to manipulate insemination (Sota, 2002). Therefore, the genetic basis of the carabid genitalia involving few loci may facilitate coevolution and the species-specific correspondence of functional parts in male and female genitalia. If the locus number for female genitalia is actually smaller than that for male genitalia, this may represent a result of more selection (due to female choice and male sex drive) on genes for male traits than on those for female traits, as postulated by Singh and Kulathinal (2005). However, more research for the numbers of loci determining male and female genital characters in the carabid beetles is required.

The evolutionary trend in the morphology of the copulatory piece in *Ohomopterus* has been inferred based on nuclear gene genealogy (Sota and Vogler, 2003); ancestral species of *Ohomopterus* have small, triangular copulatory pieces, whereas derived species have more exaggerated shapes, either wider or shorter (as in *iwawakianus*) or narrower or longer (as in *maiyasanus*). This evolutionary pattern may be explained by independent occurrences of mutations increasing the width of the copulatory piece (CPW) and mutations increasing the length (CPL). Although some genetic correlation may exist between CPW and CPL because these dimensions unlikely increase simultaneously, the diversification in copulatory piece may have been facilitated by various combinations of alleles at multiple loci, with the effect of expanding or elongating the copulatory piece.

In *Ohomopterus*, the shapes and sizes of the male copulatory piece and female vaginal appendix show concerted diversification across species and subspecies. This concerted diversification of male and female traits may have resulted from tight linkage between the loci determining male and female genital morphologies or pleiotropic regulation due to a common genetic background. This study does not provide any information regarding location of loci in the genome or intersexual association of loci. It would therefore be reasonable to initiate QTL mapping, which provides fundamental information such as the number of loci involved in a certain trait (morphological or behavioural), locations on chromosomes, and magnitude of individual genetic effects. QTL mapping with wild populations is generally difficult because of the need for large populations for mapping or interspecific hybridization experiments (Erickson *et al.*, 2004). However, several recent studies with a variety of organisms have indicated a promising future for QTL mapping (Hawthorne and Via, 2001;

Parsons and Shaw, 2002; Kronforst *et al.*, 2006). Our results provide a strong platform for further work on the genetic basis of carabid genital morphology using a QTL mapping approach.

Acknowledgements

References

Albertson RC, Streelman JT, Kocher TD (2003). Genetic basis of adaptive shape differences in the cichlid head. *J Heredity* **94**: 291–301.

Arnqvist G (1989). Sexual selection in a water strider: the function, mechanism of selection and heritability of a male grasping apparatus. *Oikos* **56**: 344–350.

Arnqvist G (1998). Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**: 784–786.

Arnqvist G, Danielsson I (1999). Copulatory courtship, genital morphology and male fertilization success in water striders. *Evolution* **53**: 147–156.

Danielsson I, Askenmo C (1999). Male genital traits and mating interval affect male fertilization success in the water strider *Gerris lacustris*. *Behav Ecol Sociobiol* **46**: 149–156.

Eberhard WG (1985). *Sexual Selection and Animal Genitalia*. Harvard University Press: Cambridge.

Erickson DL, Fenster CB, Stenoien HK, Price D (2004). Quantitative trait locus analyses and the study of evolutionary process. *Mol Ecol* **13**: 2505–2522.

Hatfield T (1997). Genetic divergence in adaptive characters between sympatric species of stickleback. *Am Nat* **149**: 1009–1029.

Hawthorne DJ, Via S (2001). Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* **412**: 904–907.

Hosken DJ, Stockley P (2004). Sexual selection and genital evolution. *Trends Ecol Evol* **19**: 87–93.

House CM, Simmons LW (2003). Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc R Soc Lond B* **270**: 447–455.

House CM, Simmons LW (2005). The evolution of male genitalia: patterns of genetic variation and covariation in the genital sclerites of the dung beetle *Onthophagus taurus*. *J Evol Biol* **18**: 1281–1292.

Huttunen S, Aspi J (2003). Complex inheritance of male courtship song characters in *Drosophila virilis*. *Behav Gen* **33**: 17–24.

Ishikawa R (1987). On the function of the copulatory organs of *Ohomopterus* (Coleoptera, Carabidae, genus Carabus). *Kontyu* **55**: 202–206.

Ishikawa R (1991). The evolution of Carabus (in Japanese). Yasaka-shobo: Tokyo.

Jagadeeshan S, Singh RS (2006). A time-sequence functional analysis of mating behaviour and genital coupling in *Drosophila*: role of cryptic female choice and male sex-drive in the evolution of male genitalia. *J Evol Biol* **19**: 1058–1070.

Kronforst MR, Young LG, Kapan DD, McNeely C, O'Neill RJ, Gilbert LE (2006). Linkage of butterfly mate preference and wing color preference cue at genomic location of *wingless*. *Proc Natl Acad Sci USA* **103**: 6575–6580.

Lande R (1981). The minimum number of genes contributing to quantitative variation between and within populations. *Genetics* **99**: 541–553.

Liu J, Mercer JM, Stam LF, Gibson GC, Zeng Z-B, Laurie CC (1996). Genetic analysis of a morphological shape difference

in the male genitalia of Drosophila simulans and D. mauritiana. *Genetics* **142**: 1129–1145.

Lynch M, Walsh B (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates: Sunderland, MA.

Mather K, Jinks JL (1982). *Biometrical Genetics. The Study of Continuous Variation*, 3rd edn. Cambridge University Press: Cambridge.

Otto SP, Jones CD (2000). Detecting the undetected: estimating the total number of loci underlying a quantitative trait. *Genetics* **156**: 2093–2107.

Parsons YM, Shaw KL (2002). Mapping unexplored genomes: A genetic linkage map of the Hawaiian cricket *Laupala*. *Genetics* **162**: 1275–1282.

Saldamando CI, Miyaguchi S, Tatsuta H, Kishino H, Bridle JR, Butlin RK (2005). Inheritance of song and stridulatory peg number divergence between *Chorthippus brunneus* and *C. jacobsi*, two naturally hybridizing grasshopper species (Orthoptera: Acrididae). *J Evol Biol* **18**: 703–712.

Serrano J, Galián J (1998). A review of karyotypic evolution and phylogeny of carabid beetles (Coleoptera). In: Ball GE, Casalle A, Taglianti AV (eds). *Phylogeny and Classification of Caraboidea (Coleoptera: Adephaga)*. Museo Regionale di Scienze Naturali: Torino. pp 191–228.

Shaw KL (1996). Polygenic inheritance of a behavioral phenotype: interspecific genetics of song in the Hawaiian cricket genus *Laupala*. *Evolution* **50**: 256–266.

Singh RS, Kulathinal RJ (2005). Male sex drive and the masculinization of the genome. *BioEssays* **27**: 518–525.

Sota T (2002). Radiation and reticulation: extensive introgressive hybridization in the carabid beetles *Ohomopterus* inferred from mitochondrial gene genealogy. *Popul Ecol* **44**: 145–156.

Sota T, Kubota K (1998). Genital lock-and-key as a selective agent against hybridization. *Evolution* **52**: 1507–1513.

Sota T, Vogler AP (2003). Reconstructing species phylogeny of the carabid beetles *Ohomopterus* using multiple nuclear DNA sequences: heterogeneous information content and the performance of simultaneous analyses. *Mol Phylogenet Evol* **26**: 139–154.

Takami Y (2003). Experimental analysis of the effect of genital morphology on insemination success in the ground beetle *Carabus insulicola* (Coleoptera: Carabidae). *Ethol Ecol Evol* **15**: 51–61.

Takami Y, Sota T (in press). Rapid diversification of male genitalia and mating strategies in *Ohomopterus* ground beetles. *J Evol Biol*.

Wenninger EJ, Averill AL (2006). Influence of body and genital morphology on relative male fertilization success in oriental beetle. *Behav Ecol* **17**: 656–663.

Westerbergh A, Doebley J (2002). Morphological traits defining species differences in wild relatives of maize are controlled by multiple quantitative trait loci. *Evolution* **56**: 273–283.

Wijngaarden PJ, Brakefield PM (2000). The genetic basis of eyespot size in the butterfly *Bicyclus anynana*: an analysis of line crosses. *Heredity* **85**: 471–479.

Zeng Z-B (1992). Correcting the bias of Wright's estimates of the number of genes affecting a quantitative character: a further improved method. *Genetics* **131**: 987–1001.

Zeng Z-B, Houle D, Cockerham CC (1990). How informative is Wright's estimator of the number of genes affecting a quantitative character? *Genetics* **126**: 235–247.

Zeng Z-B, Liu J, Stam LF, Kao CH, Mercer JM, Laurie CC (2000). Genetic architecture of morphological shape difference between two *Drosophila* species. *Genetics* **154**: 299–310.