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Interspecific difference and frequency dependence in habitat use of coexisting *Ohomopterus* ground beetle species under reproductive interference

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Abstract

In reproductive interference, the fitness of individuals or populations is decreased through reproductive interactions with other species. This process results in positive frequency dependence, hindering species coexistence. However, theory predicts that species can coexist under weak reproductive interference. Habitat segregation can decrease the opportunity for reproductive interactions between species. Thus, a difference in habitat preference between species may weaken reproductive interference and facilitate coexistence. We examined this hypothesis by investigating the habitat uses of closely related *Ohomopterus* ground beetle species under reproductive interference, *Carabus insulicola* and *C. esakii*, in limited zones of sympatry at their distributional boundary. The effect of reproductive interference may be stronger for females of *C. esakii* than those of *C. insulicola* due to asymmetry in the genital size mismatch between the species (*C. insulicola* males have larger genitalia). Field surveys of local abundances and associations with local environmental parameters revealed contrasting habitat uses between the species. *C. insulicola* preferred open environments, while *C. esakii* inhabited forest environments. Interestingly, the habitat use of *C. esakii*, not *C. insulicola*, changed depending on the frequency of the other species; the species utilized habitats with a low frequency of *C. insulicola*. The difference in habitat use and its dependence on the frequency of the other species may facilitate species coexistence by promoting habitat segregation within a continuous landscape. Our findings provide insights into the importance of (plastic) trait differences in species distributions and coexistence under reproductive interference.

KEYWORDS

Carabus, coexistence, genitalia, habitat segregation, hybridization

1 | INTRODUCTION

Reproductive interference (RI) is a process in which the fitness of individuals or populations is decreased through reproductive interactions with other species (Grether et al., 2017; Gröning & Hochkirch, 2008; Kyogoku, 2015; Mitchell et al., 2022; Suker & Burdfield-Steel, 2017). RI causes positive frequency dependence, and relatively less frequent species experience stronger impacts of RI (Kuno, 1992; Ribeiro & Spielman, 1986; Yamamichi et al. 2023; Yoshimura & Clark, 1994). The positive frequency dependence of RI predicts the exclusion of less frequent species, hindering the coexistence of species with reproductive interactions. RI also predicts selection against individuals likely to experience interference, which can lead to trait differentiation between species and a subsequent decrease in the impact of RI (i.e., reproductive character displacement; Brown & Wilson, 1956; Howard, 1993). Trait differentiation may enable species coexistence under weak RI (Kyogoku & Sota 2017). Characterization of the dynamics of distributional boundaries between closely related species with reproductive interactions can clarify the processes determining the distribution, exclusion, and coexistence of species under RI.

Most theories of RI do not account for spatial context, while some predict exclusive or parapatric distributions (Ribeiro & Spielman, 1986), niche differentiation between species (Crowder et al., 2011; Nishida, 2015; Ruokolainen & Hanski, 2016; Kyogoku 2024), or the importance of habitat segregation in species isolation and coexistence (Kyogoku & Kokko, 2020; Kyogoku & Yamaguchi, 2023). A related theory also predicts the coexistence of ecologically equivalent species under sexual selection (sexual isolation) and spatially-varied habitat capacity (M'Gonigle et al.,

2012). A species contact zone (or hybrid zone) is an arena of reproductive interaction between species (Barton & Hewitt, 1985), in which the spatial structure of the habitat may be a crucial determinant of RI. In this context, differentiation in habitat preference may play a role in the distribution of species under RI. It can result in the segregation of (micro)habitats, leading to patchy distributions in species contact zones (mosaic hybrid zone; Harrison, 1990). Such spatial isolation may decrease the encounter rate and opportunity for reproductive interactions, even within a continuous landscape (Bridle et al., 2001). Patches occupied by single species may serve as source populations, and individuals from different patches may encounter each other after dispersal and co-occur at the margins of habitats. These processes may facilitate the co-occurrence of species at their distributional boundaries even under RI (i.e., species coexistence within a landscape). Additionally, since positive frequency dependence is a key determinant of individual fitness under RI, a change in habitat use in response to the frequency of the other species may be favored, possibly decreasing the impact of RI. To examine these hypothetical dynamics of species distributions and coexistence under RI, we evaluated (1) the difference in habitat preference among species and (2) the frequency dependence of their habitat use.

Ground beetle species belonging to the subgenus *Ohomopterus* Reitter (genus *Carabus* Linnaeus) are ideal for examining the impact of RI on species distributions. This group of flightless insects is endemic to the Japanese Archipelago. It consists of about 15 species, with remarkable diversity in body size and genital morphology (Fujisawa et al., 2019; Sota et al., 2000). Up to four *Ohomopterus* species with different body sizes can coexist (Sota et al., 2000). The body size difference between males and females in interspecific mating is a good predictor of male conspecific mate choice,

mounting, and genital insertion, in which RI is more likely to be hindered when the body size difference is large (Okuzaki et al., 2010; Okuzaki & Sota, 2018). By contrast, premating isolation is often incomplete between species with similar body sizes (Kubota et al., 2013; Okuzaki et al., 2010). Interspecific mating often results in a serious decrease in female fitness. *Ohomopterus* species have a species-specific genital lock-and-key system, involving a sclerotized copulatory piece in the male and membranous receptacle vaginal appendix in the female (Ishikawa, 1987, 1991, Nishimura et al., 2022). A mismatch between male and female genitals typically injures females and decreases their reproductive output (Kubota et al., 2013; Sota & Kubota, 1998). Note that this type of fitness loss due to physical injury is not rescued by subsequent conspecific mating, as in the Satyr effect (Ribeiro & Spielman, 1986), unlike other types of RI (e.g., Noriyuki et al., 2012). Thus, interspecific mating and the resultant female fitness loss can be a major mechanism underlying RI, influencing the possibility of species coexistence. As a result, species with similar body sizes with frequent interspecific mating exhibit exclusive and mostly parapatric distributions (Sota, 2002; Okuzaki et al., 2010; Takami & Osawa, 2016). Additionally, RI may be more important than resource competition in determining species distributions and coexistence. The larvae of *Ohomopterus* species commonly and exclusively feed on earthworms belonging to Megascolecidae, suggesting resource competition. However, the signature of resource partitioning among coexisting species has not been detected, suggesting that resource competition is not a primary constraint on species coexistence (Okuzaki et al., 2010).

Carabus insulicola Chaudoir and *C. esakii* Csiki are closely related *Ohomopterus* species distributed in the eastern Honshu mainland in the Japanese

Archipelago. *C. insulicola* is broadly distributed in the northeastern part (i.e., Tohoku, Kanto, and Chubu districts), while *C. esakii* is rather limited to the southwestern part (i.e., the western part of Kanagawa Prefecture and eastern Shizuoka and Yamanashi prefectures). These species are largely parapatric but form limited sympatric zones at their distributional boundaries (Figure 1) (Ishikawa & Ujiie, 2000: Ujiie et al., 2010). The sympatric zones of *C. insulicola* and *C. esakii* are much wider (up to ca. 40 km) than those of other pairs of parapatric species with narrow hybrid zones at their boundary (a few kilometers wide, Kubota & Sota, 1998, Takami & Suzuki, 2005) and are also wider than expected based on the dispersal ability of beetle individuals (ca. 10 m per day, Kubota, 1996). However, the factors contributing to their coexistence are unclear. Despite a body size difference between the species (female body length: *C. insulicola*, ca. 30 mm; *C. esakii*, ca. 25 mm, Figure S1), males mate indiscriminately with females of the other species (Kubota et al., 2013), resulting in time and energy costs. Additionally, since these species have divergent genital morphologies, interspecific mating reduces female reproductive fitness, especially in *C. esakii* females (which have smaller genitalia and are likely to be injured by the larger male genitalia of *C. insulicola*) (Kubota et al. 2013). These facts suggest that RI occurs when the two species are in contact. However, it is unclear how and why sympatric zones are maintained at their distributional boundaries under RI. Here, we performed planned field surveys to examine: (1) whether habitat preferences differ between species in their sympatric area, and (2) whether and how their habitat uses depend on the frequency of the other species. We discuss the role of interspecific differences in habitat preference in species coexistence under RI.

2 | MATERIALS AND METHODS

2.1 | Field survey

Field surveys were conducted in the area of sympatry of *C. insulicola* and *C. esakii* (Figure 1), large- and medium-sized species. Two additional *Ohomopterus* species, *C. albrechti* Morawitz and *C. lewisianus* Breuning, occur in the study area; they are small, closely related, and show parapatric distributions (Takami & Ishikawa, 1997; Takami & Suzuki, 2005). These two species belong to the most ancestral lineage in *Ohomopterus* with small genitalia (Fujisawa et al., 2019) and are unlikely to interact reproductively with other relatively large-sized species (Okuzaki et al., 2010; Sota & Sasabe, 2006). We treated these two small species as a single species and included them in analyses to examine their possible influence on the focal species.

Field surveys were performed during the reproductive season (May to June) of *Ohomopterus* ground beetles in 2011–2013. We chose four localities (localities 1 to 4) where the two target species potentially co-occur (Figure 1, Table S1). These localities consisted of forest (dominated by cultivated Japanese cedar *Cryptomeria japonica* and deciduous oaks *Quercus* spp.), grassland, and their margins. Locality 4 was surveyed twice, both in 2012 and 2013. We treated the two surveys in the locality 4 separately because of annual variation in environmental factors (Figure 2) and species occurrence (Table S2), resulting in five sets of surveys in total (Table S1). In each survey, two to seven sites were established along a transect from forest to open grassland to capture environmental variation in the habitat (Table S1). Sites on the transect were separated by at least 5 m. Each site consisted of 20 emptied pitfall traps (65 mm in diameter, 90 mm in depth) with 2 m intervals, arranged in a line perpendicular to the transect. As a

result, field data were obtained from 4 localities, 5 surveys, 21 sites, and 420 traps. Twenty-four hours after setting the traps, beetles captured at each site were counted for each species and sex.

Three environmental parameters were measured at each site on the day of setting traps. Soil temperature was measured on the ground surface to the nearest 0.1°C in the morning (ca. 8:00 to 11:30 a.m.) using an infrared thermometer (IT-330, Horiba). Litter depth near the trap was measured using a vernier scale to the nearest 0.5 cm. Canopy openness was calculated based on a hemispherical photograph taken with a digital camera (Coolpix 4500, Nikon) equipped with a fish-eye lens (FC-E8, Nikon) using CanopOn2 (http://takenaka-akio.org/etc/canopon2/). In principle, canopy openness ranges from 0% (completely closed) to 100% (completely opened). We measured these parameters at 3–5 arbitrarily chosen points along the trap line at a site. These measurements were averaged within a site and used in the following analysis.

2.2 | Analysis of body size variation

The body size difference between species is an important determinant of RI in ground beetles (Okuzaki et al. 2010; Okuzaki & Sota, 2018). Thus, the intensity of RI may vary among localities in response to geographical variation in body size. To examine this possibility, we examined body size variation among surveys and sites. Beetles collected at each site were fixed with ethyl acetate, then pinned, and dried. Body length (distance from the anterior margin of the labrum to the apices of the elytra) was measured to the nearest 0.01 mm using digital calipers. General linear models (normal distribution) were constructed for each species, with individual body length as the

objective variable. Survey, site nested within survey, and sex were included as explanatory variables.

2.3 | Principal component analysis of environmental data

The three environmental parameters were evaluated by a principal component analysis because they were correlated with each other (soil temperature vs. litter depth, $r_{19} = -0.39$, $P = 0.084$; soil temperature vs. canopy openness, $r_{19} = 0.67$, $P = 0.001$; litter depth vs. canopy openness, $r_{19} = -0.45$, $P = 0.039$). The correlation matrix was decomposed to generate three orthogonal axes (envPCs) using the *prcomp* function in R (Table S3). envPC1 explained 67.1% of the total variance and was characterized by positive loadings of soil temperature and canopy openness, and by a negative loading of litter depth (Figure 2), indicating transitional environmental variation from the forest floor to open grassland. envPC2 explained 21.9% of the total variance and was characterized by a strongly negative loading of litter depth (Figure 2). envPC3 explained 10.9% of the total variance and was characterized by a strong positive loading of soil temperature and a strong negative loading of canopy openness. We used envPC1 and envPC2 as environmental variables in subsequent analyses (89.1% of variance explained).

2.4 | Analysis of habitat use

First, we checked for sex differences in habitat use. Generalized linear mixed models (GLMMs) with a log-link and Poisson distribution were constructed for each species, with the number of males or females collected at each site as the objective variable. Sex, envPC1, and envPC2 as well as interaction terms between sex and

envPCs were included as explanatory variables. The survey was included as a random term, assuming spatial and temporal variation among five surveys fits a normal distribution. A significant interaction between sex and envPCs was expected if habitat uses differ between sexes.

Second, we examined whether habitat uses differ between species. A GLMM was constructed with the total number of individuals (sum of males and females) collected at each site as the objective variable. The use of the total number of individuals was justified based on the lack of or little difference in habitat use between the sexes (see Results). Species, envPCs, and interaction terms between species and envPCs were included as explanatory variables. Survey was included as a random term. A significant interaction was expected if habitat uses differ between species. Then, we characterized the habitat use for each species. GLMMs were constructed using datasets for single species, with the number of individuals collected at each site as the objective variable. envPC1 and envPC2 were included as explanatory variables and survey as a random term.

Third, we were interested in whether and how the habitat use of a species depends on the frequency of the other species. The GLMMs for each species were modified by adding the frequencies of the other two species and their interactions with envPCs as explanatory variables. The frequency of another species was calculated for each site as (individuals of another species)/(individuals of all three species + 1), where 1 was added to the denominator to avoid zero values. Note that the denominator of this measure includes the objective variable, indicating that this measure can be correlated with the objective variable. Instead of this coefficient, we were interested in interactions between the frequency of another species and envPC. A significant interaction was

expected if habitat use changed depending on the frequency of the other species. GLMMs were fit using the *glmer* function in the *lme4* package (Bates et al., 2015), and the type II Wald test was applied for multi-categorical variables using the *Anova* function of the *car* package (Fox et al., 2019) in R. Prediction curves were calculated based on GLMMs and plotted using the *plot_model* function in the package *sjPlot* (Lüdecke et al., 2023).

3 | RESULTS

In five surveys, we collected 131 individuals of *Ohomopterus* ground beetles, consisting of 23 individuals (11 females and 12 males) of *C. insulicola*, 88 (53 females and 35 males) of *C. esakii*, 16 (12 females and 4 males) of *C. lewisianus,* and 4 (2 females and 2 males) of *C. albrechti* (Table S2). Although the localities were chosen based on the possible co-occurrence of *C. insulicola* and *C. esakii*, *C. insulicola* was not collected in surveys 1 and 2. *Carabus insulicola* and *C. esakii* cooccurred at four sites (one site in survey 3 and three sites in survey 5). *Carabus insulicola* and *C. albrechti* cooccurred at three sites (survey 5). *Carabus esakii* and *C. lewisianus* or *C. albrechti* cooccurred at eight sites (four in survey 1, one in survey 2, and three in survey 5).

Adult body length differed significantly among surveys for *C. insulicola* and *C. esakii*, suggesting that geographical variation in body size may contribute to RI (Table S4, Figure S1). Note that we accounted for this variation in the analyses of habitat use by including survey as a random term. Body length also differed significantly among sites for *C. insulicola* (Table S4). Females were significantly larger than males in the three species (Table S4, Figure S1).

In analyses of sex differences in habitat use, we did not detect significant interactions between sex and envPCs for *C. insulicola,* and for *C. lewisianus* and *C. albrechti* (Table S5). However, for *C. esakii*, we detected a marginally significant interaction between sex and envPC2 ($P = 0.045$) (Table S5), in which positive envPC2 coefficients were shared by the sexes but stronger in males (GLMM for males, envPC2, 2.84 ± 0.75, $z = 3.79$, $P = 0.0001$; GLMM for females, envPC2, 0.86 ± 0.33, $z = 2.58$, $P = 0.0099$). Thus, we summed the numbers of males and females and used the total number of individuals of each species per site in subsequent analyses.

The observed habitat uses differed among the species. In the GLMM including the data for the three species, the interaction between species and envPC1 was significant ($\chi^2 = 31.23$, d.f. = 2, $P < 0.0001$), while that between species and envPC2 was not ($\chi^2 = 0.13$, d.f. = 2, $P = 0.94$). In the GLMM for *C. insulicola*, envPC1 showed a significant positive association with the number of individuals, indicating a preference for open environments, while envPC2 was nonsignificant (Table 1, Figure 3a). The scatter plot showed no apparent association with the presence/absence of *C. esakii* (Figure 3a). By contrast, in the GLMM for *C. esakii*, envPC1 showed significant negative association with the number of individuals, indicating a preference for forest environments (Table 1, Figure 3b). Although the scatter plot for *C. esakii* indicated that the number of individuals peaked at an intermediate envPC1 value, this variation corresponded to the presence/absence of *C. insulicola* (Figure 3b) and was explained by the frequency of *C. insulicola* in the following analysis (see below). For *C. esakii*, envPC2 also showed a significant positive association, indicating a preference for shallow litter. In the GLMM for *C. lewisianus* and *C. albrechti* in combination, envPC1 showed significant negative association with the number of individuals, indicating a

preference for forest environments, while envPC2 was not significant (Table 1, Figure 3c).

The habitat use observed in *C. esakii* depended on the frequencies of other species; a similar frequency dependence was not observed in *C. insulicola*, and in *C. lewisianus* and *C. albrechti*. For *C. esakii*, all four interaction terms between envPCs and the frequency of *C. insulicola*, as well as between envPCs and the frequency of *C. lewisianus* and *C. albrechti*, were significant (Table S6). The negative association between envPC1 and the number of individuals per site (i.e., preference for forest environments) was strengthened in the presence of *C. insulicola* (Figure 4), indicating increased habitat segregation between species. The use of forest habitats became more conspicuous in the absence of *C. lewisianus* and *C. albrechti* (Figure 4), indicating the utilization of vacant forest environments. Frequency-dependent changes in habitat use regarding envPC2 in *C. esakii* were also significant but difficult to interpret because preferences for envPC2 were not significant in the other species (Table 1).

4 | DISCUSSION

As theory predicts that RI is frequency-dependent, empirical studies often assume sexual exclusion and resultant spatial segregation between species under RI. Although these assumptions have been useful for detecting RI in the wild (e.g., Noriyuki et al., 2012; Takakura & Fujii, 2010, 2015), they have also precluded studies of species coexistence under RI, which is theoretically possible, especially when habitats are spatially segregated. Our results provide empirical evidence for species coexistence under RI in the wild within a heterogeneous environment.

We detected contrasting habitat uses between *C. insulicola* and *C. esakii,* which are likely to experience RI in sympatry (Table 1, Figure 3). This suggests difference in habitat preference, which may lead to habitat segregation, decrease interspecific interactions, and facilitate species coexistence within a continuous landscape. Relatively large-sized *C. insulicola* preferred open environments, while relatively small-sized *C. esakii* utilized forest environments. This association between body size and habitat type was also consistent with the use of forest environments in the very small *C. lewisianus* and *C. albrechti* (Figure 3). Since a large body may be advantageous in arid environments, as seen in ground beetles in the genus *Pamborus* (Sota et al., 2005), the observed association between body size and habitat type may be a result of adaptative differentiation to variation in habitat wetness (i.e., dry open habitats vs. wet forest habitats). This could explain the observed body size variation among sites in *C. insulicola*. A study of contact zones between another pair of *Ohomopterus* species also indicated that habitat wetness is a major factor determining distributional boundaries (Takami & Osawa, 2016). Thus, spatial heterogeneity in wetness may be an important factor for the coexistence of *C. insulicola* and *C. esakii*.

It is unclear whether RI promoted the differentiation in habitat preference (i.e., reproductive character displacement) because our dataset was obtained from only the area of sympatry. To examine reproductive character displacement, habitat preferences need to be compared between sympatric and allopatric regions. In addition, the observed difference in habitat use between species could be a behavioral response to interspecific interactions (discussed below in detail). However, it is unlikely that the interspecific difference in habitat preference can be explained by plasticity alone (i.e., behavioral) because the habitat use of *C. insulicola* in open environments was

independent of the frequencies of the other species (Table S6), and because the GLMM predicted that *C. esakii* utilized forest environments even after the frequencies of other two species were adjusted to zero (Figure 4).

The habitat use in *C. esakii* depended on frequencies of other species, suggesting that interspecific interactions influenced habitat choice in this species. Frequency-dependent changes in habitat use in *C. esakii* were observed across sites. Sites were situated within narrow areas, and localities were also often close to each other (Figure 1). The estimated dispersal rates of adult *Ohomopterus* beetles were more than 10 m/day in a continuous habitat (Kubota, 1996), suggesting that beetles can move across sites within a locality in a reproductive season and may respond to local frequencies of other species. Thus, frequency dependence in the habitat use of *C. esakii* may have a behavioral (i.e., plastic) basis and therefore may not be due to genetic differentiation among sites. Some models of individual movement may be relevant to this (e.g., Ruokolainen & Hanski, 2016). Observed frequency-dependent changes in habitat use were directed towards increasing habitat segregation or utilizing vacant environments (Figure 4). Thus, in addition to differences in habitat preference between species, plasticity in habitat use can also decrease the opportunity for ecological and reproductive interaction with other species and facilitate their coexistence. This finding is relevant to the importance of plastic trait changes in species coexistence under RI (Kyogoku & Wheatcroft, 2020). However, our results cannot discriminate between the effects of ecological competition and RI, although these processes are not mutually exclusive and can interact with each other (Kyogoku & Kokko, 2020). For *C. esakii* and *C. insulicola*, there is solid evidence for RI (Kubota et al., 2013), suggesting that RI plays a role, at least to some extent. By contrast, for *C. lewisianus* and *C. albrechti*,

there is no direct evidence for RI with other two species, making detailed interpretation of the results is difficult.

Why was a frequency-dependent change in habitat use only observed for *C. esakii*? This may be explained by an asymmetry in RI. Due to the interspecific difference in genital sizes (Figure 1), the probability of injury is higher for *C. esakii* females with small-sized genitalia mated with *C. insulicola* males with large-sized genitalia than for *C. insulicola* females mated with *C. esakii* males (Kubota et al. 2013). Thus, *C. esakii* females may suffer a relatively greater decrease in reproductive fitness in interspecific mating with *C. insulicola* males, suggesting that the impact of RI is greater for *C. esakii*. In this situation, a frequency-dependent change in habitat use may allow *C. esakii* females to avoid the fitness loss by decreasing the encounter rate with *C. insulicola* males. *C. esakii* males may be less likely to experience this type of fitness loss than females, and the frequency-dependent change in habitat use may be less important for males than in females, although males need to search for conspecific females (Kyogoku & Yamaguchi, 2023). A slight sex difference in habitat use regarding envPC2 in *C. esakii* may reflect a difference in the impact of RI between the sexes. This result can also be explained by sample sizes. Since *C. esakii* was the most abundant species in our surveys, the detection of associations with explanatory variables may be more likely. Our dataset was rather small, warranting further studies with more replications over wider geographical scales.

In conclusion, we examined the processes contributing to species coexistence under RI using closely related *Ohomopterus* ground beetle species in limited sympatric zones at their distributional boundary. We found interspecific differences in habitat use and its dependence on the frequency of other species. These differences may facilitate

species coexistence by promoting habitat segregation within a continuous landscape. Our findings provide insights into the importance of (plastic) trait differences in species distributions and coexistence under RI.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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TABLE 1 Generalized linear mixed models explaining the number of individuals per site. Significant results are shown in boldface ($p < 0.05$). envPC1 and envPC2 refer to the first and second principal components based on three environmental variables, respectively.

	Carabus insulicola			*C. esakii*			*C. lewisianus* and *C. albrechti*		
	Estimate ± SD	*z*	*p*	Estimate ± SD	*z*	*p*	Estimate ± SD	*z*	*p*
Intercept	-1.035 ± 1.058	-0.98	0.3276	1.059 ± 0.299	3.54	**0.0004**	-1.305 ± 0.753	-1.73	0.0830
envPC1	0.486 ± 0.229	2.12	**0.0339**	-0.747 ± 0.123	-6.06	**<0.0001**	-0.787 ± 0.320	-2.46	**0.0138**
envPC2	0.292 ± 0.293	1.00	0.3182	0.562 ± 0.141	3.98	**<0.0001**	0.319 ± 0.300	1.06	0.2886

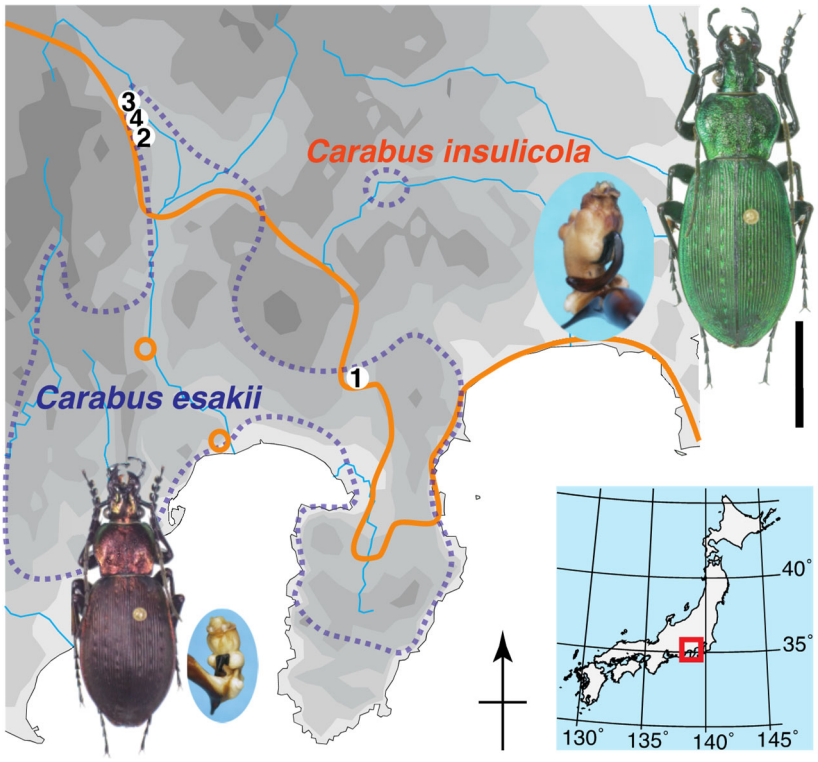

FIGURE 1 Distributional map of *Carabus insulicola* (orange solid line) and *C. esakii* (purple dotted line) in central Japan (red rectangle in lower right inset). Numbers (1–4) indicate localities for the field survey. External habitus and male genital morphologies are also shown. Scale bar is 10 mm for bodies and 5 mm for genitalia.

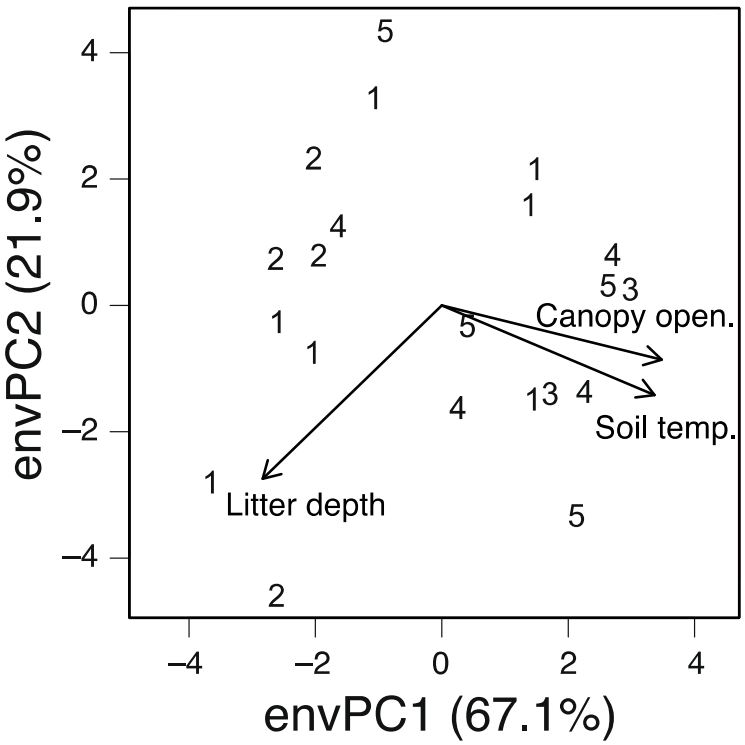

FIGURE 2 Principal component analysis of three environmental variables. envPC1 and envPC2 refer to the first and second principal components, respectively. Numbers correspond to sites in surveys (1–5).

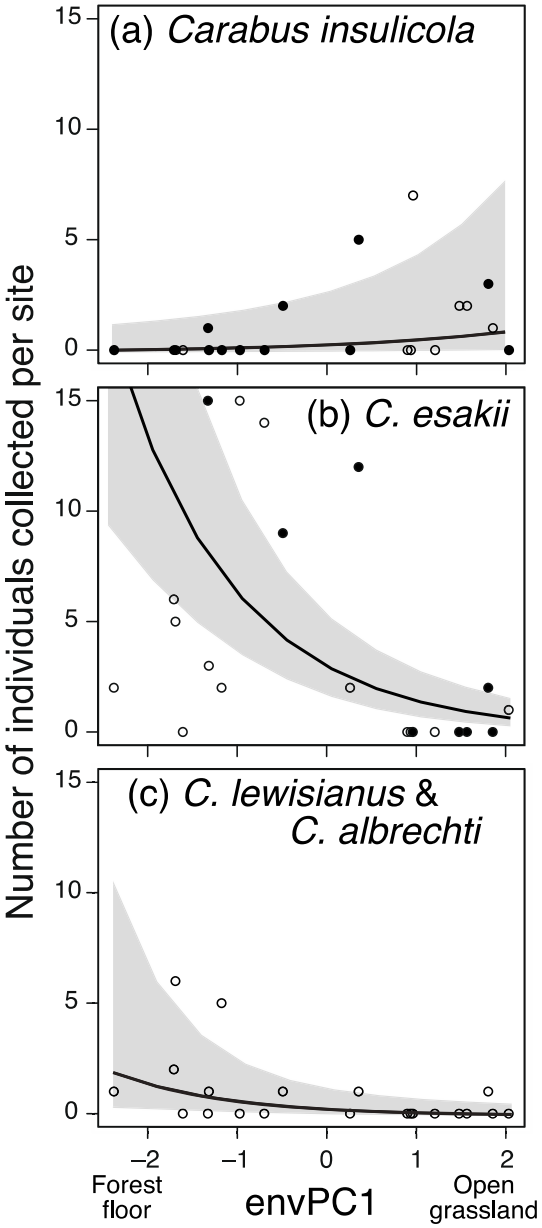

FIGURE 3 Association between the environmental gradient from forest to grassland (envPC1) and the number of individuals collected per site in (a) *Carabus insulicola*, (b) *C. esakii*, and (c) *C. lewisianus* and *C. albrechti*. Filled and open circles in (a) and (b) refer to the presence and absence of the related species (*C. esakii* in (a) and *C. insulicola* in (b)), respectively. Curves and 95% prediction intervals are shown based on GLMMs (Table 1).

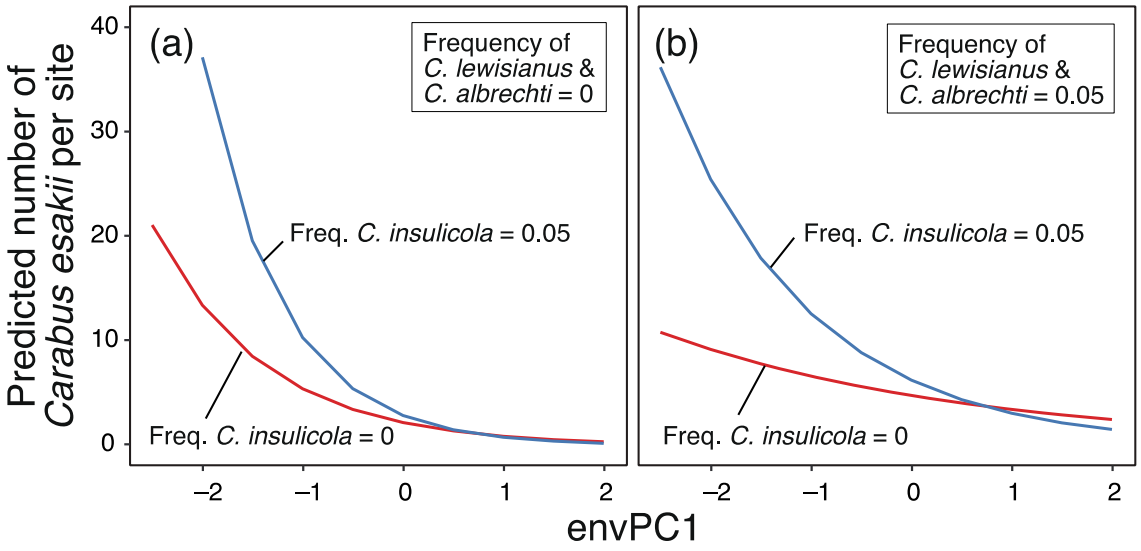

FIGURE 4 Interaction plots showing the habitat use of *Carabus esakii* depending on the frequency of other species: (a) effect of the frequency of *C. insulicola* in the absence of *C. lewisianus and C. albrechti* and (b) that in the presence of *C. lewisianus* and *C. albrechti.* The frequencies of other species (=0.05) were chosen arbitrarily for visualization. Curves are estimated based on GLMMs (Table S6).