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Adaptive divergence of scaling relationships mediates the arms race between a weevil and its host plant

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Coevolution of exaggerated morphologies between insects and plants is a well-known but poorly understood phenomenon in evolutionary biology. In the antagonistic interaction between a seed-predatory insect, the camellia weevil (*Curculio camelliae*), and its host plant, Japanese camellia (*Camellia japonica*), we examined the evolutionary trajectory of an exaggerated offensive trait of the weevil (rostrum length) in terms of scaling relationship. Sampling throughout Japan revealed that the ratio of the rostrum length to overall body size was correlated with the ratio of the pericarp thickness to overall fruit size across the localities. We found a geographical interpopulation divergence in a parameter pertaining to the allometric equation of rostrum length (the coefficient a in $y=ax^b$, where y and x denote rostrum and body lengths, respectively), and the pattern of geographical differentiation in the allometric coefficient was closely correlated with the variation in the pericarp thickness of Japanese camellia. Our results provide a novel example of a geographically diverged scaling relationship in an insect morphology resulting from a coevolutionary arms race with its host plant.

Keywords: allometry; arms race; coevolution; exaggeration; geographical mosaic; predator–prey interactions

1. INTRODUCTION

Antagonistic or mutualistic interactions between insects and plants sometimes promote the coevolution of astonishingly exaggerated morphologies, such as the extremely long tongue of a *Xanthopan* hawkmoth pollinating the Madagascar star orchid with its 30 cm long spur (Darwin 1862; see also Johnson & Steiner 1997; Nilsson 1998). To date, experimental and field studies have shown that natural selection potentially favours such elaborated traits in interacting species (Nilsson 1988; Alexandersson & Johnson 2002). However, few reports have thoroughly examined the occurrence of reciprocal evolution, which should be a driving force in the escalation of interacting traits. Furthermore, few studies have attempted to reveal that coevolutionary selection has promoted the divergence of exaggerated traits among populations.

Recently, we reported a promising example of geographically structured coevolution in a system involving a seed predator, the camellia weevil (*Curculio camelliae*: Curculionidae: Coleoptera), and its host plant, Japanese camellia (*Camellia japonica*: Theaceae) (Toju & Sota 2006; see also Thompson 2005). The weevil is an obligate seed predator of Japanese camellia, and the female excavates the fruit of its host with its extremely long rostrum to oviposit in the seeds (figure 1*a*,*b*; Okamoto 1988). The Japanese camellia defends its seeds from the weevil's attacks with a very thick pericarp, and the seeds of individuals with a thicker pericarp have higher survival rates (Toju & Sota 2006). Importantly, the rostrum length of the female weevil shows remarkable geographical variation (9–19 mm), which is clearly correlated with variation in the pericarp thickness of Japanese camellia (6–20 mm; Toju & Sota 2006). These facts suggest that the exaggerated traits of the two species are involved in an arms race (Dawkins & Krebs 1979), and the coevolutionary process is escalated in some populations (coevolutionary hotspots), but not in others (coevolutionary coldspots; Thompson 1999).

An exaggeration of defensive/offensive morphology may simply be based on a shared scaling relationship (Labarbera 1989; Emlen & Nijhout 2000), such that the rostrum length of the weevil, a putative target of coevolutionary selection, increases with overall body size following a single allometric equation (i.e. $y=ax^b$; see Gould 1966). However, because of natural selective pressures acting on body size *per se* (e.g. Masaki 1967), the exaggeration in the rostrum may have required alterations in the scaling relationship among local populations under differential selection regimes on the coevolutionary trait. To clarify the adaptive divergence of traits involved in coevolutionary processes, it is therefore crucial to explore the differentiation of scaling relationships for the focal traits. Although several studies have demonstrated the response to selection in scaling relationships of exaggerated traits in insects (Wilkinson 1993; Emlen 1996), the interpopulation differentiation in the parameters of scaling relationships has seldom been explored (cf. Swallow *et al.* 2005).

Here, we show that the allometric features of camellia weevils can diverge remarkably among populations as a result of coevolution with Japanese camellia. First, we examine the concordance in geographical patterns of exaggeration between the rostrum length of the weevil and the pericarp thickness of the plant by investigating the interpopulation variation in the scaling of those traits against overall body or fruit sizes. Second, we test whether the scaling relationship of the weevil's rostrum length to overall body size significantly diverges among populations. Concomitantly, we examine the correlation between the geographical variation in allometric coefficients of weevil rostrum and pericarp thickness of Japanese camellia to examine whether the evolution of the allometric features of the weevil

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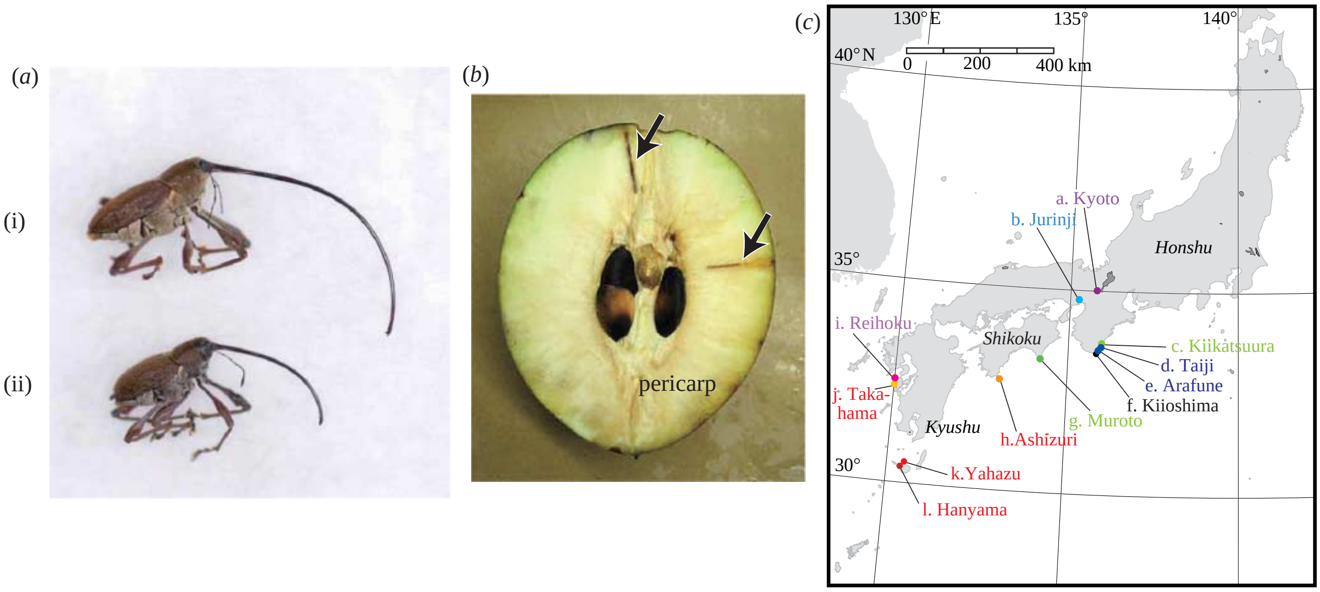

Figure 1. Study organisms and sampling localities. (*a*) The geographical variation in the rostrum length of camellia weevils (i) Hanyama; (ii) Jurinji. (*b*) A cross-section of a Japanese camellia fruit. Arrows represent holes made by weevils. (*c*) Twelve sampling localities.

rostrum occurred in response to the evolution of the defensive trait of Japanese camellia.

2. MATERIAL AND METHODS

We collected specimens of the camellia weevil and Japanese camellia from 12 populations over almost the entire range of weevil in Japan (figure 1*c*). The rostrum length and body length (head margin excluding the rostrum to the elytral apex) of female weevils were measured on images taken with a digital camera (see Toju & Sota (2006) for detailed methods) and then the ratio of rostrum length to body length (i.e. rostrum length/body length; the rostrum ratio, hereafter) was calculated for each individual. The diameter and pericarp thickness of Japanese camellia fruit were measured to the nearest 0.01 mm using digital callipers and averaged over individual trees. From the measurements, we calculated fruit radius (half of the fruit diameter) and the ratio of pericarp thickness to fruit radius (pericarp thickness/fruit radius; the pericarp ratio, hereafter) for each tree. Finally, we tested the correlation between the rostrum and the pericarp ratios.

To reveal how the scaling relationship of rostrum length against overall body size varied across populations, we performed an analysis of covariance (ANCOVA). Both traits were log-transformed before analysis, and thus the function $y=ax^b$ becomes $\log y = \log a + b \log x$, where x and y denote untransformed body and rostrum lengths, respectively (allometric equation). Using an ANOVA of rostrum length with body length, locality and the interaction term between the two traits, we tested whether slopes of regressions for individual populations were differentiated. Subsequently, the adjusted mean of rostrum length was linearly regressed on the pericarp thickness of Japanese camellia to test whether the interpopulation variation in the allometric coefficient of the rostrum was concordant with the geographical pattern of the defence of the plant. All statistical analyses were performed using JMP v. 5.01 (SAS Institute, Inc., Cary, NC).

3. RESULTS

The rostrum ratio of camellia weevils and the pericarp ratio of Japanese camellia, significantly differentiated among populations (see electronic supplementary material), were clearly correlated with each other across populations ($r=0.91$, $p<0.0001$; figure 2*a*). In an ANOVA model of rostrum length with body length, locality and their interaction effects, the interaction term was not significant (d.f.$=11$, $F=1.4$, $p=0.17$), while the effects of body length and locality were highly significant ($p<0.0001$ for both effects). Eliminating the interaction term from the model, the effects of both terms were still significant (log body length effect: d.f.$=1$, $F=587.3$, $p<0.0001$; locality effect: d.f.$=11$, $F=96.1$, $p<0.0001$). For all local populations, the common slope and intercepts (effect of localities) were then obtained to reveal the regressions of rostrum length on body length (figure 2*b*). The common slope, which corresponded to b of the allometric function, was 1.335, larger than unity (s.e.$=0.055$, 95% CI: 1.227–1.443, d.f.$=243$, $t=24.2$, $p<0.0001$). The adjusted mean of the logarithm of rostrum length, which was substituted for the intercepts of the lines and thus for log a of the allometric equation, varied greatly among populations and increased with the pericarp thickness of Japanese camellia (*adjusted mean*$=0.7053+0.3642\times$(log *pericarp thickness*), $N=12$, $F=105.4$, $p<0.0001$; figure 2*c*).

4. DISCUSSION

A geographical correlation was observed between the scaling relationship of the weevil rostrum and the Japanese camellia pericarp (figure 2*a*). Furthermore, both the rostrum and the pericarp ratios were larger in southern populations (see electronic supplementary material), in which directional selection on the pericarp thickness of the plant was stronger (Toju & Sota 2006). These results support the hypothesis that the two species are involved in a geographically structured coevolutionary arms race (Toju & Sota 2006).

From the analysis of allometric coefficients, we found that the coefficient b of the allometric equation ($y=ax^b$) was not geographically differentiated and was significantly greater than unity (cf. Swallow *et al.* 2005). The allometric function with $b>1$ revealed a pattern that rostrum length of the weevil increased exponentially as body length increased, and more importantly, female weevils possessed longer rostra on average in southern populations (Toju & Sota 2006), where the mean of their body sizes was larger (Toju & Sota in press).

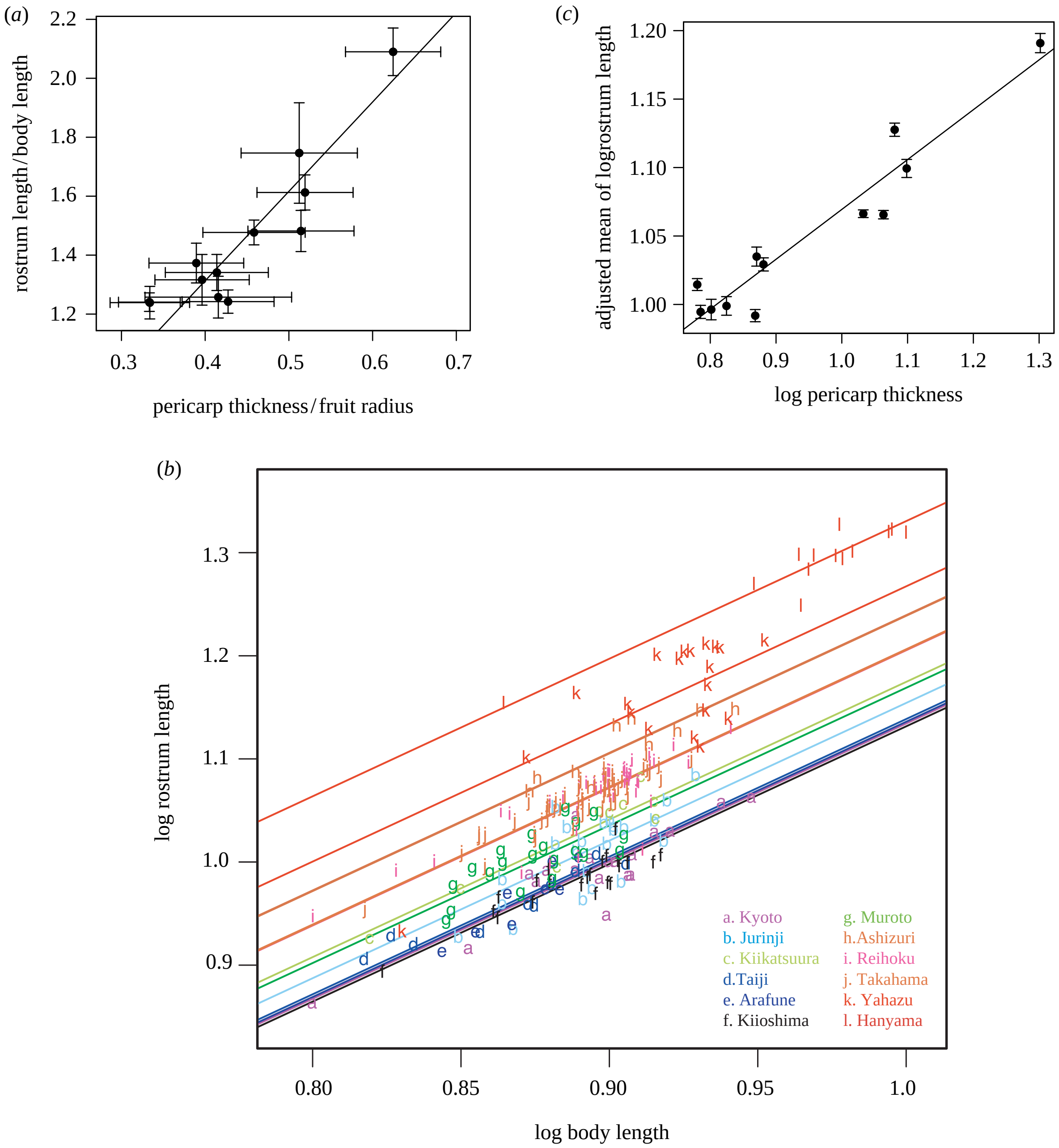

Figure 2. The geographical divergence of scaling relationships. (*a*) The correlation between the rostrum and pericarp ratios across populations (±s.d.). (*b*) ANCOVA for the rostrum length of the camellia weevil. Characters represent localities (see also figure 1). (*c*) The correlation between the pericarp thickness of Japanese camellia and the allometry of the rostrum length of the camellia weevil across populations. The allometric coefficient of the weevil is substituted by the adjusted mean of rostrum length calculated from ANCOVA (±s.e.).

A successive test indicated that coefficients of locality (i.e. logarithms of a) were significantly differentiated; thus, weevils from different populations but with the same body size were expected to have different lengths of rostra (figure 2*b*). This interpopulation variation in the allometric coefficient a resulted in the differentiation of scaling relationships and caused substantial geographical variation in rostrum length. In summary, two properties of allometric equations were important for the camellia weevil to have realized its exaggerated rostrum in the south: the b term was larger than unity and increased body sizes in these populations, and an increase occurred in the a term. These investigations are essential for understanding the evolutionary trajectory of exaggerated insect traits because recent advances in theoretical and developmental biology allow us to infer how the shapes of allometric equations have changed in relation to alternation in developmental processes (Nijhout & Wheeler 1996; Emlen & Nijhout 2000; Emlen & Allen 2004).

Furthermore, the allometric coefficient a, which is represented by the adjusted mean of the logarithm of rostrum length, was clearly correlated with the pericarp thickness of Japanese camellia (figure 2*c*). This result was also concordant with the hypothesis that the allometry of rostrum length has evolved to overcome the defences of Japanese camellia. Although examples that show the adaptation of insect morphologies to the defences of host plants (Hughes & Vogler 2004; Carroll *et al.* 2005) exist, our results are the first to demonstrate that geographically structured coevolution can cause substantial intraspecific variation in the allometric equations of exaggerated insect traits. Therefore, our results indicate that the geographic mosaic of species interactions (Thompson 1999) provides an excellent framework for determining the trajectory of escalating coevolution.

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