Evidence of Trait Shifts in Response to Forest Disturbance in Taiwanese *Carabus masuzoi* (Coleoptera: Carabidae)

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Abstract

Shifts of functional traits are important because phenotypic responses of species to environmental changes caused by natural and anthropogenic disturbances are fundamental in determining the risk of population extinction. This study tested the effect of forest thinning on the body shape and male genital size of an endemic ground beetle species *Carabus masuzoi* (Imura and Satô 1989) (Coleoptera, Carabidae) in cypress plantations started approximately 30 yr ago in central Taiwan. The beetles were sampled and compared from 1) natural broadleaf forest, 2) non-thinned cypress plantation, and 3) 45% thinned cypress plantation. Female prothorax length from the non-thinned plantation was significantly greater than that of the natural forest, and the 45% thinned plantation had a higher frequency of the small (S-type) and a lower frequency of the large (L-type) male genitalia than in the natural forest. The results indicated that, within a short ecological time frame, the prothorax shapes and male genital sizes of *C. masuzoi* populations might respond to changes induced by different forest types and forest thinning, respectively. We hypothesized that the difference in prothoracic shape was related to locomotory ability in forest understories, whereas the changes in male genital sizes might have been a result of different levels of intraspecific sexual selection, random effects of population fluctuations/dispersal, or pleiotropy.

Key words: body shape, cypress plantation, functional trait, ground beetle, male genitalia

Natural and anthropogenic disturbances can alter habitat conditions, subsequently eliminating local populations of poorly adapted species and significantly changing the abundance and distribution of resources for surviving populations (Schowalter 2012). The anthropogenic disturbance of forest thinning is a frequently used management strategy to restore the health and commercial value of plantation forests and to meet the demands of ecosystem services and the conservation of biodiversity (Hobbs and Norton 1996, Stanturf et al. 2014). Among Asian plantation forests, many studies have documented that changes in the plant community and environmental characteristics caused by forest thinning can shape the species richness, abundance, and community structure of arthropod communities (e.g., Ohsawa and Nagaike 2006; Taki et al. 2010; Huang et al. 2011, 2014). Studying the changes in community structure caused by forest thinning thus provides a summary of the impact of this disturbance. But the use of diversity indices alone may overlook important information associated with the ecologically meaningful (functional) traits of individual species. This information is important because the phenotypes of organisms are the direct targets of natural selection, and specific phenotypic responses of species to environmental changes caused by disturbances are fundamental in determining the risk of population extinction (Chevin et al. 2010).

Ground beetles (Carabidae) are frequently used as ecological indicators of forest ecosystems because of their abundance for easy sampling, sensitivity to human-altered conditions, and relatively stable taxonomy for efficient identification and long-term monitoring (Rainio and Niemelä 2003, Pearce and Venier 2006, Koivula 2011). They can respond quickly to habitat fragmentation through changes in their species abundance and richness via either the dispersal of generalists or the recolonization of habitat specialists (Niemelä 2001, Rainio and Niemelä 2003). In terms of specific phenotypic effects of disturbance, carabids have been shown to either increase or decrease in body size after being disturbed by a hurricane (Garbalinska and Skłodowski 2008), and their body sizes decreased with increasing levels of heavy metal pollution (Lagisz 2008). More brilliant metal coloration and smaller sizes were found in carabids inhabiting disturbed farmland than those in pine forests, which could reflect a recent adaptation to the changed environment or random genetic
drift (Tyler 2010). Forest thinning and ground vegetation cutting changed the wing types (brachypyrg vs macroptermy) of carabids, which might be linked to vegetation structural instability favoring macropterous beetles after the disturbance (Shibuya et al. 2014). However, the current knowledge regarding the specific effects of forest thinning on the phenotypic traits of individual carabid species is still limited.

*Carabus* (*Apotomopterus*) *masuzoi* (Imura and Satô 1989) (Coleoptera, Carabidae) is an endemic ground beetle species distributed in mountain forests between approximately 1,800 and 2,500 m in Taiwan. This species can disperse between habitats only through running because of its highly reduced hind wings (brachypyrgous). As a part of a larger-scale study to examine the effects of forest thinning on the invertebrates of cypress plantation forests in Taiwan (e.g., Huang et al. 2011, 2014), the aim of this study is to verify whether anthropogenic disturbance of the forest ecosystem (i.e., forest thinning) may contribute to the modification of the body shape and genital morphology of *C. masuzoi*. This carabid species was chosen because of its relatively high abundance in the study area (approximately 60% of all collected carabids) and its limited dispersal capabilities. An earlier study correlating the morphological and life history traits of carabids with land disturbance indicated that, in highly disturbed lowland sites, species were smaller and had higher dispersal ability (Ribera et al. 2001). Therefore, more disturbed forests (higher degree of thinning) would be expected to have smaller individuals with morphologies associated with a higher dispersal ability (such as broader bodies and longer legs) (Evans 1994). The diverse morphology of male genitalia in carabids is often related to different levels of intraspecific sexual selection (Takami 2003, Takami and Sota 2007). We tested the following specific hypothesis: forest thinning induces changes in the body shape and the frequencies of male genital sizes in *C. masuzoi* populations.

**Methods**

**Sampling of Ground Beetles**

Beetle sampling was carried out in five consecutive seasons (September and November of 2005; February, May, and August of 2006) in a cypress plantation (*Chamaecyparis formosensis*) on Mt. Da-Shiue of central Taiwan (24°15′40″ N, 120°59′18″ E; altitude between 1,800 and 2,000 m). This cypress plantation was established approximately 30 yr ago and underwent long-term rotational thinning practices. The overall density of this cypress plantation was about 900–1,800 trees/ha. A neighboring broadleaf forest (next to the 45% thinned cypress plantation) of mostly beeches, oaks, and laurels was included as a control. This broadleaf forest was selected to minimize the climatic and topological difference between the forests; however, the movement of ground beetles between these sampled forests is possible. Nine sampling plots (each separated by 50 m) were established in the center of each of three types of cypress forests: 1) natural broadleaf forest (900–1,800 trees/ha, >>10 ha); 2) non-thinned cypress plantation (1,200 trees/ha, 8 ha); and 3) 45% thinned cypress plantation established in 2004 (825 trees/ha, 10 ha) (a total of 27 sampling plots). A randomized block design was used to spatially assign the forest types (Lee and Yen 1995). The distance of each sampling plot to the edge of the forest was more than 100 m to avoid edge effects. For each plot, a set of pitfall traps was used to collect samples for a week. Each set of pitfall traps consisted of four plastic cups (10 cm diameter and 15 cm depth) filled with 500 ml of 70% alcohol, with three plastic plates (100 cm length and 30 cm width) arranged in a Y-shape. The more detailed location of the studying sites and the sampling scheme of the experiments were described in Huang et al. (2011).

**Measurement and Statistical Analysis**

The collected specimens were dissected to remove appendages and genitalia. The body was secured with insect pins onto a Styrofoam plate and then dried in an oven at 50°C for 72 h. One hundred and twenty (67 males and 53 females) out of 247 collected beetle specimens were used for the analysis of genitalia due to the quality of specimen preservation in the field and the failure of dissection and preparation of the genitalia in the laboratory (Supp. Appendix 1 [online only]). Male genitalia were bathed in 15% KOH at 65°C for 20 min to clear the structure. The endophallus of the male genitalia was injected with toothpaste (DARLIE, Hawley & Hazel Chemical Co., Taipei, Taiwan) using a syringe and then dried at 50°C for 2 h. This process was repeated twice to completely expand the endophallus. Female genitalia were kept in 50% glycerin. Three male genital types can be recognized based on the morphological differences in the endophallus (Fig. 1d): 1) L-type, the parapraepatual lobe is longer
than the preapertual pad; 2) M-type, the parapapertual lobe and preapertual pad are equal in length; and 3) S-type, the parapapertual lobe is shorter than the preapertual pad. The chi-square statistic was used to test the significance of the deviation in the frequencies of the male genital types in the non-thinned and 45% thinned plantations from the expected frequencies based on the natural forest. Three photographs (JPEG at 300 dpi) of the dorsal and ventral body (head, thorax, and abdomen), the lateral male genitalia, and the female genitalia were taken using a digital camera (D90, Nikon, Tokyo, Japan) with a micro lens (105 mm DG MACRO lens, f 2.8, SIGMA, Kanagawa-ken, Japan) and a white light projected from above at an angle of 45°. The length (mm) of the body (head [HL], prothorax [PTL], and abdomen [ABL]) (Fig. 1a), aedeagus (AL) (Fig. 1c), and female genitalia (L1 and L2) (Fig. 1e) were obtained using the photographs in PHOTOSHOP CS3 (Adobe Systems Inc., San Jose, CA). The measurement error (%ME) was calculated by comparing the variation within and between individuals based on a formula developed by Bailey and Byrnes (1990). The measurement errors were 15.8% (head), 16.9% (thorax), 16.0% (abdomen), 13.9% (aedeagus), and 3.8% and 8.0% (female genitalia L1 and L2, respectively). We used the Kolmogorov–Smirnov test to examine the normality of the distribution of the measurements, and then used one-way analysis of variance (ANOVA) to test the significance of the differences among the forest types. Non-normally distributed measurements were analyzed using the Kruskal–Wallis test. Tukey’s HSD (honest significant difference) test was conducted when the above two tests were significant. All statistical analyses were conducted using SPSS v. 17.0 (Norusis 2005).

Geometric Morphometrics

We selected 49 landmarks at the junction or extreme of anatomical structures (head 13, thorax 19, abdomen 17; Fig. 1b) in tpsDig2 (Rohlf 2005) to quantify the body shape for geometric morphometric (GM) analysis. The coordinates of the landmark locations were transformed into eigenvectors using general Procrustes analysis (GPA) (Rohlf and Slice 1990) in CoordGen6 of the Integrated Morphometric Package (IMP) (Sheets 2004) and then summarized using the canonical variate analysis (CVA) in CVAGen6k of IMP. The shape variables (uniform components and relative warps) of body shape were used as dependent fixed variables and the centroid size as a covariate in multivariate analyses of covariance (MANCOVA) to statistically evaluate the body shape differences among the forest types. Thin-plate spline deformation grids were generated between the consensus of the body shapes of the three forest types in CVAGen6k to visualize the direction and level of deformation.

Results and Discussion

We obtained 247 C. masuzoi specimens from the three forest types (natural forest, 94; non-thinned plantation, 70; 45% thinned plantation, 83). The ANOVAs for the body measurements indicated that they were similar among the males of the three forest types (Table 1). The female PTL (prothorax length) of individuals from the non-thinned plantation was significantly greater than that of those from the natural forest, whereas the female PTL of individuals from the 45% thinned plantation was not different from that of individuals from either the non-thinned plantation or the natural forest. Nevertheless, the difference in PTL of the females is small, and we recognize that due to multiple comparisons there was a greater possibility to encounter Type I errors (corrected significant level < 0.025). The analyses of genital sizes (AL, L1 and L2) revealed no significant difference among individuals of the three forest types. These results indicated that the body size of C. masuzoi did not change in response to heavy forest thinning (45%) but that forest type (natural vs plantation) might affect the prothoracic size of females.

The GM analyses of the body shape demonstrated significant sexual dimorphism in all body parts (MANCOVA, P < 0.006), but a significant difference in the body shape among the forest types was found only in the thorax (MANCOVA, Wilk’s lambda = 0.374, F = 0.487, P = 0.023). The combination of thoracic shape variables (CV1 and CV2) showed little overlap among the C. masuzoi of the three forests (Fig. 2a and b). The deformation grid showed that the majority of the thoracic shape changes occur in the anterior coxal cavities (Fig. 2c–f). For example, compared to the natural forest, the males in the non-thinned plantation showed a posterior extension of their coxal cavities (landmarks 8, 9, 10, and 11), while their prothorax expanded anteriorly (landmarks 16, 17, 18, and 19) (Fig. 2c). Because the body shape (morphology) of carabids is likely to dictate their locomotory performance to some extent (Evans 1994), the difference in prothoracic size and shape of C. masuzoi between the natural forest and cypress plantation might be related to their running, pushing, or digging ability in forest understory areas, where the natural forest has higher understory vegetation density and dry litter weight than that of the non-thinned and thinned plantations (Huang et al. 2011). Nevertheless, whether or not these subtle morphological changes affect the locomotory ability of C. masuzoi needs to be tested.

Table 1. ANOVAs for the measurements (mean ± sd, mm) of body sizes of Carabus masuzoi from different forest types (HL, head length; PTL, prothorax length; ABL, abdomen length; TL, total length; AL, aedeagus length; L1, L2, female genitalia length; Fig. 1e)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Body parts</th>
<th>Natural forest</th>
<th>Non-thinned plantation</th>
<th>45% thinned plantation</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>HL</td>
<td>3.36 ± 0.22</td>
<td>3.35 ± 0.17</td>
<td>3.30 ± 0.19</td>
<td>0.938</td>
<td>0.394</td>
</tr>
<tr>
<td></td>
<td>PTL</td>
<td>3.97 ± 0.15</td>
<td>3.97 ± 0.17</td>
<td>3.89 ± 0.21</td>
<td>2.668</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>ABL</td>
<td>13.91 ± 0.44</td>
<td>13.70 ± 0.46</td>
<td>13.79 ± 0.43</td>
<td>2.326</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>TL</td>
<td>21.24 ± 0.67</td>
<td>21.02 ± 0.65</td>
<td>20.98 ± 0.54</td>
<td>2.172</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td>AL</td>
<td>4.46 ± 0.13</td>
<td>4.50 ± 0.14</td>
<td>4.44 ± 0.15</td>
<td>–</td>
<td>0.901</td>
</tr>
<tr>
<td>Female</td>
<td>HL</td>
<td>3.54 ± 0.26</td>
<td>3.63 ± 0.21</td>
<td>3.55 ± 0.17</td>
<td>1.903</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>PTL</td>
<td>3.91 ± 0.18</td>
<td>4.03 ± 0.17</td>
<td>3.93 ± 0.18</td>
<td>5.238</td>
<td>0.025*</td>
</tr>
<tr>
<td></td>
<td>ABL</td>
<td>14.60 ± 0.60</td>
<td>14.54 ± 0.52</td>
<td>14.54 ± 0.38</td>
<td>0.145</td>
<td>0.865</td>
</tr>
<tr>
<td></td>
<td>TL</td>
<td>22.04 ± 0.90</td>
<td>22.20 ± 0.82</td>
<td>22.03 ± 0.63</td>
<td>0.605</td>
<td>0.547</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>5.37 ± 0.55</td>
<td>5.33 ± 0.69</td>
<td>5.45 ± 0.57</td>
<td>0.168</td>
<td>0.846</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>1.28 ± 0.10</td>
<td>1.27 ± 0.10</td>
<td>1.31 ± 0.06</td>
<td>0.623</td>
<td>0.544</td>
</tr>
</tbody>
</table>

*AL was not normally distributed, thus the Kruskal–Wallis test was used.
*PTL was significantly different among the different forests, and the Tukey’s HSD test was employed (*P ≤ 0.05).
experimentally by measuring their running speed or strength in pushing/digging across various understory vegetation types.

The frequency of male genital sizes was significantly different between the natural forest and the 45% thinned plantation (Table 2). The 45% thinned plantation had a higher frequency of the small (S-type) genitalia and a lower frequency of the large (L-type) genitalia than in the natural forest, whereas the frequencies were similar between the natural forest and the non-thinned plantation. This finding implies that 45% thinned forest may lead to changes in the frequency of male genital sizes in *Carabus masuzoi* populations, which might have been a result of different levels of intraspecific sexual selection between the two habitats, or due to the random effects of population fluctuations and dispersal between habitats. Intraspecific sexual selection for driving the difference in male genital sizes may be possible given this short time frame (approximately 30 generations), as shown in an experimental population of dung beetles, *Onthophagus taurus* (testes size, 21 generations; Simmons and García-González 2008). In addition, the pleiotropic effect resulted from genetic correlation between genitalia and other body parts may possibly be responsible for the differentiation of genital morphology (Hosken and Stockley 2004).

Because the evolution of the genital morphology of male carabids has been considered to improve insemination success by depositing spermatophores at the proper site for sperm transfer into the spermatheca of the female (Takami 2003), further studies evaluating the functional significance of male genital sizes in *C. masuzoi* and estimating the level of intrasexual selection in different forest types are required to test these hypotheses. Future sampling in these cypress plantations, which are under long-term rotational thinning practices, can be conducted to determine if similar patterns of phenotypical changes (e.g., smaller male genitalia in recently thinned forests) can be detected.

This study showed that the shapes of prothorax and the sizes of male genitalia of *C. masuzoi* populations might respond to changes induced by different forest types and heavy forest thinning, respectively. These preliminary results suggest that these two functional traits essential for locomotion and reproduction in carabids may change substantially over a short ecological time scale of only 30 yr after the disturbance. The other phenotypic traits (such as body size) might not be sensitive to habitat alterations, which might be due to opposing selective forces or the physiological and developmental constraints of this species. The phenotypic responses might be related to

### Table 2. Chi-square statistics for the frequencies of male genital types of *Carabus masuzoi* among different forest types

<table>
<thead>
<tr>
<th>Forest types</th>
<th>L-type</th>
<th>M-type</th>
<th>S-type</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural forest (expected)</td>
<td>37.14%</td>
<td>48.57%</td>
<td>14.29%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-thinned plantation</td>
<td>36.84%</td>
<td>47.37%</td>
<td>15.79%</td>
<td>0.189</td>
<td>0.910</td>
</tr>
<tr>
<td>45% thinned plantation</td>
<td>17.39%</td>
<td>52.17%</td>
<td>30.43%</td>
<td>18.262</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Numbers in the parentheses indicate the sample sizes from 27 sampling plots (\( ^*P \leq 0.05 \)).
specific environmental or biological changes caused by differences in habitat types and forest thinning; however, due to the limitation of the experimental design (the beetle samples not available from 30 yr ago before the forest thinning) and the problem associated with sampling scheme (pseudo-replication, the sampling plots of each forest type located within a single forest), we could not exclude the possibility that geographical and/or historical variation independent of forest types may be responsible for the observed difference in Carabus masuzoi populations. The underlying mechanisms (adaptation, between-habitat dispersal, or random genetic drift, and pleiotropy) for possible trait shifts in Carabus masuzoi populations require further investigation.

Data Availability Statement
Data from this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.p94d8 (Chen et al. 2018).

Supplementary Data
Supplementary data are available at Annals of the Entomological Society of America online.

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