Utilization of geometric morphometrics for discrimination of European spruce bark beetle (*Ips typographus* L.) populations in Western Carpathians

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Abstract. Successful biological control of pests requires detailed information about the target population. In this study we used geometric morphometrics to discriminate *Ips typographus* L. populations from 3 selected sites in Slovakia. Fourteen wing landmarks and nine elytra landmarks were used to compute shape variables. Canonical variate analysis revealed highly significant ($P < 0.0001$) differences in wing and elytra shape between beetles of different sex, generation and locality. The most pronounced shape changes were observed between the bark beetles of different sex. Geographic origin had the least but still highly significant influence on the shape of wing and elytra. Our results indicate that geometric morphometrics may be a useful tool in the assessment of interpopulation variability of spruce bark beetles.

Introduction

European spruce bark beetle (*Ips typographus* L.) is an important insect species due to the high ecological and economic impact it may have on Norway spruce forests. Recent outbreaks of the spruce bark beetle population in High Tatras following the windstorm in 2004 have risen demands to develop more effective methods to assess its population structure and dynamics.

Biological control of pests and estimation of their population status requires detailed information about the pest including differences among populations as these may influence the outcome of bio control programs. The present study evaluates utilization of geometric morphometrics for discrimination of European spruce bark beetle populations in selected sites of Slovakia.

Data and methods

Samples of 90-120 beetles were collected from pheromone traps from local wood yards in Oravská Lesná, Tatranská Lomnica and Čierny Váh, Slovakia. Samples were collected twice in Oravská Lesná and Čierny Váh (June and September 2012) and four times in Tatranská Lomnica (May, June, August, and September 2012). The beetles were killed by freezing at -20 °C. Right wing and right elytra were taken from each beetle. Wings were mounted in SWAN medium and microscopic slides were prepared and photographed. Photographs of elytrae were taken directly without mounting; side views were used in the analysis. Images were captured with Leica DFC320 camera attached to a Leica MZ 12.5 stereomicroscope with Micrometrics SE Premium for Windows 7. Each beetle was dissected to determine sex of the specimen.

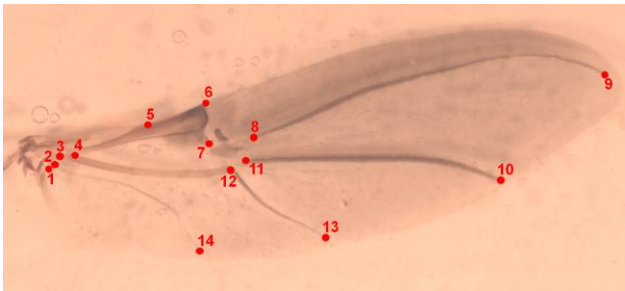
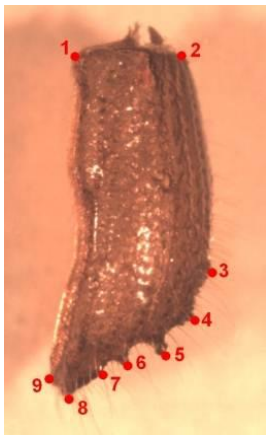


Fig. 1. Wing landmarks chosen for the analyses.

Fig. 2. Elytra landmarks chosen for the analyses.



Geometric morphometrics was used to analyze size and shape changes of the wings and elytrae with respect to the sex of the beetles, time of collection (spring or summer generation), and geographic origin. Fourteen landmarks were chosen for wings (Fig. 1) and 9 landmarks for elytrae (Fig. 2). Landmark coordinates were obtained using tpsDig v2.16 and further analyzed with MorphoJ v1.05c (Klingenberg 2011). Data were transformed with Procrustes superimposition and shape variables were subjected to canonical variate analysis (CVA) and discriminant analysis (DA). Centroid sizes were analyzed in Statgraphics v5.0. A non-parametric *t'*-test was used to estimate the effects of sex, a parametric *t*-test to estimate the effects of time of collection (generation), and Welch's ANOVA to estimate effects of geographic origin on the centroid size of wings and elytrae. A total of 502 wings and 654 elytrae were included in the analyses (damaged wings and elytrae were removed from the dataset). The dataset contained data from about twice as many females than males; this was due to a strongly shifted sex ratio of the samples collected in May and June.

Results and discussion

Overall, the highest variability of wing shape was observed in landmark coordinates 9, 13, and 14. The most variable areas of the elytrae included landmarks 1, 3, 8, and 9.

No significant differences were observed in the centroid size of wings and elytrae with respect to sex, generation or geographic origin of the beetles ($P > 0.05$).

CVA revealed highly significant differences ($P < 0.0001$) in the wing shape between males and females. The biggest differences in shape were associated with landmarks along the long axis of the wing (landmarks 1, 2, 3, 4, 8, 9, 11, and 12). A model estimated with DA was able to correctly assign sex in 76.3 % of the beetles. Similar results were also obtained in analysis of elytra shape: highly significant differences ($P < 0.0001$) were observed in CVA, with most pronounced effect of sex on position of landmarks 4, 5, 8, and 9. A model obtained by DA was able to correctly categorize 85.93 % of the beetles.

Time of sample collection also significantly ($P < 0.0001$) affected wing and elytra shape. Differences related to spring and summer generation of the beetles were most pronounced in position of landmarks 3, 4, 8, 9, 11, 12, 13, and 14 on the wing and landmarks 4, 5, 8, and 9 on the elytra. DA correctly identified the category (spring or summer generation) in 74.5 % of the beetles based on wing shape and in 67.6 % of the beetles based on elytra shape. Values obtained in analyses of elytra shape were lower than for wing shape, suggesting a stronger influence of time on wing shape than on elytra shape. Subsequent analyses done on the subset from Tatranská Lomnica, where the samples were collected on four different occasions, showed that significant differences may exist not only between spring and summer generation of the beetles but also between beetles coming from the same generation (e. g. between samples collected in May and June). Therefore, it is quite possible that aside from the generation, there may be another time-related factor which influences wing and elytra shape and which was not considered in this study.

Statistical differences ($P < 0.0001$) were also observed among beetles of different geographic origin. Most of the variability in wings was associated with landmarks 3, 5, 8, and 14. CVA produced results with two significant canonical variates but there was a big overlap among geographical groups. Variability in the shape of elytrae was observed mainly in the position of landmarks 1, 2, 7, and 9. Only one significant variate was obtained in CVA. A very big overlap was observed among geographical groups. Values obtained in the analyses of both wing and elytra shape were much lower than those obtained in the analyses regarding sex and generation of the beetles, indicating it was the least influencing factor in the analyses.

Morphological variability in populations of *I. typographus* was previously assessed only with classic morphometric tools (Sallé et al. 2005). Although proved useful, during classic morphometric analysis a lot of shape information is lost.

Geometric morphometrics has been previously shown to be useful for discrimination of species with similar morphological features, often in combination with molecular and genetic analyses (Klingenberg 2011). This study shows, however, that this method is very sensitive and thus may be useful for comparisons of populations within the same species.

Conclusion

Wing and elytra shape was highly influenced by all of the factors assessed in this study. The most pronounced effects were attributable to sex of the beetles; the least (but still highly significant) effects were associated with geographic origin of the spruce bark beetles. With this method we were able to find differences in populations of *I. typographus* located as little as 50-100 km apart. Our results indicate that geometric morphometrics may be useful in studying intra- and interpopulation variability in spruce bark beetles and could become a helpful tool in monitoring programs.

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