

Geometric morphometric analysis of morphological variation in Bogor and Solok populations of bony lip barb (*Osteochilus vittatus*)

Imron Imron^{1,*}, Fajar Anggraeni¹, Dessy Nurul Astuti¹, Flandrianto Sih Palimirmo^{1,3}, Yogi Himawan¹, Wahyu Pamungkas¹, Huria Marnis¹, Joni Haryadi², Otong Zenal Arifin¹, and Jojo Subagja¹

¹National Research and Innovation Agency (BRIN), Jalan Raya Bogor KM. 47 Cibinong, Nanggewer Mekar, Kab. Bogor, Indonesia

²Research Institute for Fish Breeding, Jl Raya 2 Sukamandi, Patokbeusi, Subang 41263, Indonesia

³Pukyong National University, 45 Yongso-ro, Nam-gu, Busan 48513, South Korea

Abstract: Bony lip barb (*Osteochilus vittatus*) represents a freshwater species belonging to the Cyprinidae family, which is naturally widely distributed in Southeast Asia. In Indonesia, it is reported to occur widely in the inland waters of the main islands of Java, Sumatra, and Kalimantan. Geographic isolation may contribute to divergent evolution, resulting in phenotypic diversity. The present study used the geometric morphometric method to identify and describe morphological feature differences between geographically distant populations, namely Bogor (West Java) and Solok (West Sumatra). Seventy individual samples were collected from Bogor and Solok, respectively. Images of each sample were taken using a digital camera, then digitized on 17 landmark points using *tpsDig* software, followed by shape analysis using *MorphoJ* software. Results showed that Geometric Morphometric analysis could identify and distinguish the body shape of both populations. Morphologically, the Bogor population had a slender body shape, while the Solok population had a shorter and broader (stubby) body shape. A combination of landmark points forming the dorsal arch (LMs 2, 3, and 15), those forming the ventral curve (LMs 9-14), and those located in the peduncle area (LM15) contributed most to the body shape differences. Possible factors causing the observed phenomena are discussed.

1 Introduction

Morphometrics, the study of the size and shape of living organisms [1], has been implemented traditionally using linear measures such as length and width, angles, ratios, and areas [2]. While it has been used in many branches of biology, traditional morphometrics has several limitations. Some of these include the possibility of autocorrelation because many

* Corresponding author: imro005@brin.go.id

measurements start from the same point, the potential of overlapping due to measurements in the same direction, and the small amount of information concerning shape [3]. More importantly, traditional morphometric data do not contain information about geometric structure, which is central to shape analysis. The shape information is an important variable but is hard to quantify. Typically, shape information is linked with size, and many procedures for removing the size effect have been introduced in the traditional morphometric method (TM) to extract the shape information [3]. Nevertheless, different standardization of procedures usually results in less consistent results. The geometric morphometric approach (GM) has addressed many of these limitations. The GM uses data on the spatial arrangement of landmarks in either two- or three-dimensional spaces along a biological structure [2]. The landmark coordinates contain information on a relative position, which is essential to morphologists. Information collected by coordinates can be partitioned into component size and shape, and landmark coordinates can be visualized informatively through graphical presentation [3].

The bony-lipped barb, *Osteochillus vittatus* (Valenciennes 1842), is a freshwater fish species belonging to the family Cyprinidae that is naturally widely distributed in Southeast Asia [4]. In Indonesia, it is reported to occur widely in the inland waters of the Indonesian main islands, such as Java, Sumatra [5], and Kalimantan [6]. A recent study reported it also occurred in Madura [7], an island next to the eastern Java mainland. It represents an economically important freshwater food fish in Indonesia, particularly in West Java and West Sumatra. Bogor in the West Java province and Solok in West Sumatera province are geographically distant sites where *O.vittatus* populations occurred. A distance of around 1300 km spanned the two locations. Additionally, the Sunda Strait, separating Java and Sumatra, has been a geographic barrier separating the two populations.

In addition to being geographically widely distributed, it also inhabits various habitats differing in its environment variables, such as elevation, water and air temperature, and bottom substrate. For instance, *O. vittatus* in West Sumatra was found in natural and artificial water bodies such as lakes, rivers, and reservoirs. They inhabited habitats with elevations ranging from 100-400 m above sea level (asl), temperatures ranging from 25 to 28°C, and various bottom substrates such as mud, rocks, or gravels [8]. In West Java, *O vittatus* was also reported to be found in areas of diverse elevation ranging from 300-700m asl [9]. Little information is available on whether geographic isolation and varying habitats have affected morphological features in *O. vittatus*. Several studies revealed that geographic isolation without gene flow may promote reproductive isolation, leading to speciation. Geographically isolated populations may develop differences in morphological features due to the interactive effect of environments, selection, and genetics on individual ontogeny [10].

A study in the genus *Hyalela* amphipod [11] found that geographic isolation plays an essential role in the evolution of reproductive isolation and divergent morphology. A similar result was also found in a study with a freshwater crustacean, *Aegla plana*, which found that variation in carapace shape was associated with biogeographic effects [12]. In contrast, a study in *Coris julis* (Teleostei, Labridae) [13] found that environmental factors have driven morphological variation of the lower pharyngeal jaw between populations. Although both environments were geographically close (around 50 km), highly different environmental variables have forced both populations to develop significant structures of the lower pharyngeal jaw. Owing to these circumstances, exploring how morphological features in *O. vittatus* of Bogor and Solok have evolved is interesting. The present study aimed to identify and describe those morphological features, specifically in the body shape differences, using the geometric morphometric method.

2 Materials and methods

2.1 Fish specimen

Specimens used in this study represent live fish, which are the collection of the Research Institute for Fish Breeding, Sukamandi, Indonesia. Two populations of *O. vittatus* were collected from Bogor, West Java province (6° 38' 39" S, 107° 10' 30" E) and Solok, West Sumatera province (01°35.626' S, 101°09.347' E). Both locations are around 1300 km away and are separated by the Sunda Strait dividing Java and Sumatra islands (Figure 1). A total of 76 fish, consisting of 66 and 10 individuals representing Bogor and Solok populations, respectively, were used in this study.



Fig. 1. Map of Indonesia (insert) showing the sampling sites of Bogor (West Java) and Solok (West Sumatera) (A) and images of morphotype of Bogor (B) and the Solok (C) populations.

2.2 Image acquisition and data collection

The images of all samples in lateral view were taken using a digital camera. Data in the form of coordinate landmarks in two dimensions (2D), as shown in Figure 2, were collected. The landmark points included 1) mouth tip at the upper lip, 2) junction between posterior cranial and anterior dorsal region, 3) anterior origin dorsal fin, 4) the anterior origin of anal fin, 5) anterior origin of ventral fin, 6) junction between ventral anterior and lower operculum, 7) anterior tip of pectoral fin, 8-16) ventral curve starting from anterior ventral fin to the operculum, and 17) posterior end of linea lateralis. Seven of the 17 landmarks were traditional: anatomical structures homologous among individuals. Points 8-18 were semi-landmarks to capture possible morphological variation along that curve. Due to the possibility of getting the inconsistent placement of landmark points, several potential homologous landmarks, such as the posterior end of the dorsal fin, anal fin, and ventral fin, were digitized. The landmark coordinates are digitized using applications *tpsDig2* and *tpsutil* [14].

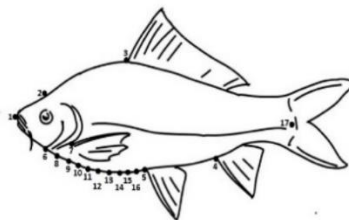


Fig. 2. Landmarks position on bony lip barb.

2.3 Data analysis

The data were then analyzed to describe and detect possible morphological variability between the two populations. The non-shape components, which consist of variations in size, position, and orientation of the samples, were removed using Procrustes superimposition [15], resulting in exclusively shape information. The shape variables were then used for further analyses. A Principal component analysis (PCA) was used to explore significant features of shape variation and discover the relationship patterns among samples through an ordination method. Discriminant function analysis was applied to compare body shape differences between the two populations. Additionally, shape changes of each population relative to the mean shape were visually displayed in a graphical presentation. These analyses were implemented in *MorphoJ*, an integrated software package for geometric morphometrics [16].

3 Results and discussion

Two landmarks (LM6 and LM7) were dropped during statistical analyses, leaving only 15 LMs. Significant features of shape variation in the data explored by PCA are presented in Figures 3 and 4. The first four components out of 26, namely PC1 to PC4, explained 77.68% of the total shape variation in the data (Table 1). The PC scores of individuals along that axis, in conjunction with the LM points that characterize that axis, are presented in Figures 3 and 4. PC1 axis accounted for 41.99% of the total variation and is characterized by a set of LMs contributing to a whole-body shape change. The LMs forming dorsal arch (LMs 1, 2, 3, and 14) and LM set reflecting ventral curve, particularly the LMs 5, 9, 10, 11, 12, and 13, which in combination created body width, all had significant coefficients. Additionally, the PC1 is also characterized by a shorter peduncle.

Combining these three body parts: higher dorsal arch, lower ventral curve, and shorter peduncle formed a stubby body shape. A PC2, which explained an additional 14.52% variance, is characterized by a higher dorsal anterior, a body structure formed by LMs 1, 2, and 3, and again a shorter peduncle. PCA loadings of LMs along the transformed axes are not shown. A scatter plot of individuals' PC scores against these two major PCs, which cumulatively explained around 55% total variation, clearly shows the clustering of the population. Plotted against PC1 and PC2, 100% and 90% of the Solok population reside on the positive side, meaning they have a higher value than the population average. Different from the Solok population, a nearly equal number of Bogor population resided on both sides of the PC1.

Table 1. Eigenvalues, percentage of relative and cumulative explained variance.

PC	Eigenvalues	% Variance	Cumulative %
1.	0.00069772	41.994	41.994
2.	0.00024122	14.518	56.512
3.	0.00019536	11.759	68.271
4.	0.00015629	9.407	77.678
5.	0.00009520	5.730	83.408
6.	0.00008806	5.300	88.708
7.	0.00005958	3.586	92.293
8.	0.00004945	2.976	95.270
9.	0.00003632	2.186	97.456
10.	0.00001585	0.954	98.410
11.	0.00001099	0.662	99.071
12.	0.00000482	0.290	99.362
13.	0.00000273	0.164	99.526
14.	0.00000241	0.145	99.671
15.	0.00000200	0.120	99.791
16.	0,00000130	0,078	99,869
17.	0,00000057	0,034	99,904
18.	0,00000054	0,033	99,936
19.	0,00000043	0,026	99,962
20.	0,00000022	0,013	99,975
21.	0,00000011	0,006	99,981
22.	0,00000009	0,005	99,987
23.	0,00000008	0,005	99,991
24.	0,00000006	0,004	99,995
25.	0,00000005	0,003	99,998
26.	0,00000003	0,002	100,000

The PC3 and PC4, which, in combination, added 21% of the total variation, were characterized by sets of LMs that reflect a high variation in dorsal structure. In PC3, the PC coefficient of LM3, connected with LM2 and LM14, formed the dorsal structure, was significant and positive, meaning that individuals within the population have a higher dorsal structure than the population average. A similar case occurred to PC4. The LM3, which was connected to LM2, formed a dorsal anterior structure, which was also significant and positive. Plotting individual PC scores against these two axes again showed a distinct grouping. While a nearly equal number of the Bogor population reside on both sides (positive and negative) of the PC3 axis, 90% of the Solok population resides on the positive side. A similar pattern was observed when individual PC scores were plotted against PC4, in which all Solok populations reside on the positive axis, meaning that this population had a higher dorsal structure.

A complementary and supporting analysis using the Discriminant function is shown in Figure 5. Figure 5A shows that the Bogor population differed in body shape from the Solok population. In general, the LMs points forming body width and length, which combined result in body shape information, characterized the differences. Viewed from a lateral perspective, the Solok population had a broader body width, as indicated by a higher position of LMs forming the dorsal arch (LMs1, 2, and 3) and a lower position of LMs forming the ventral curve (LMs 9-14), relative to the Bogor population. In addition, from a horizontal perspective, the Solok population was also slightly shorter, as indicated by a smaller value of LM15. Figure 5B clearly shows the orientation of the changes in body shape between these two intraspecific populations.

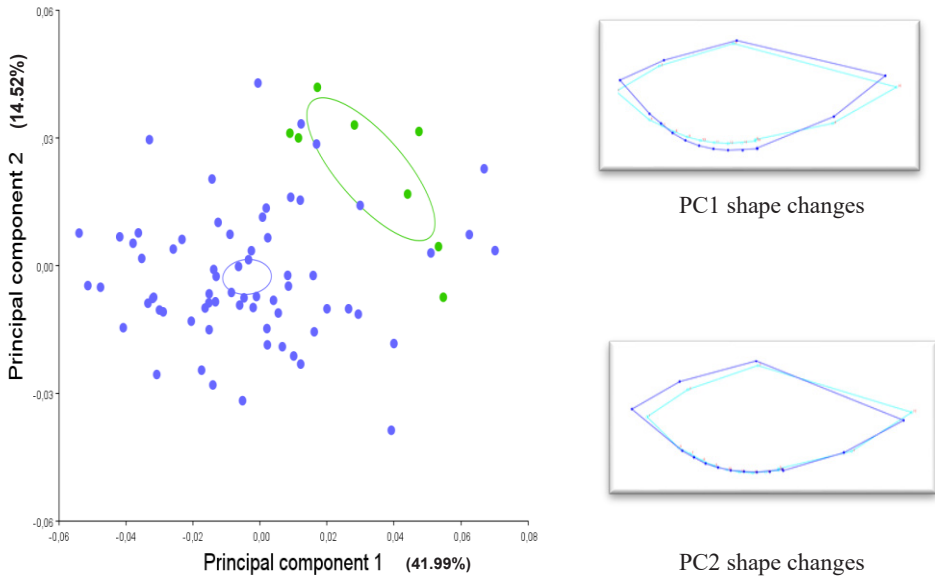


Fig. 3. Plot of individual PC scores against PC1 and PC2 axes (left side) and corresponding shape changes depicted in wireframe graph presentation (right side). Individual scores plotted in green represent the Solok population, while those in blue represent the Bogor population. Wireframe graph printed in Tosca color represents mean shape while those in blue represent shape change.

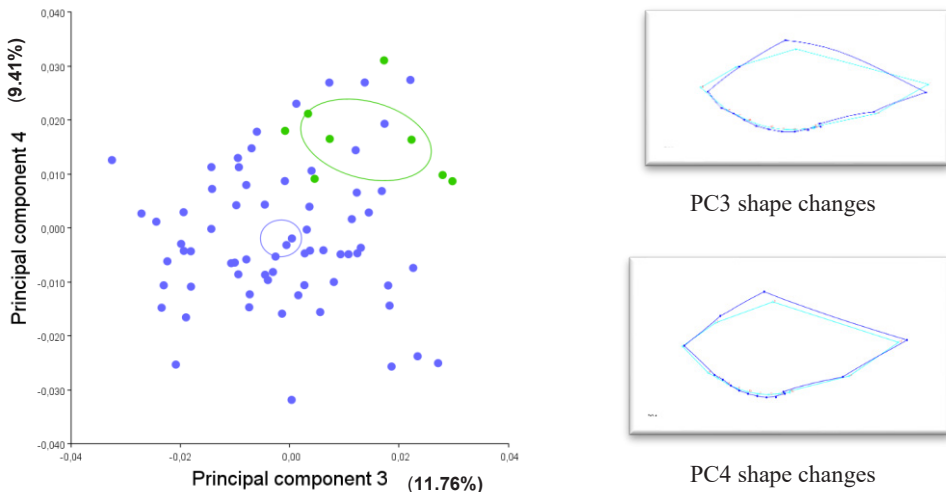


Fig. 4. Plot of individual PC scores against PC3 and PC4 axes (left side) and corresponding shape changes depicted in wireframe graph presentation (right side). Individual scores plotted in green represent the Solok population, while those in blue represent the Bogor population. Wireframe graph printed in Tosca color represents mean shape while those in blue represent shape change.

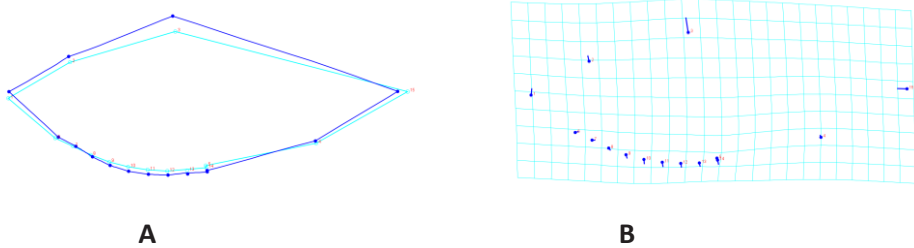


Fig. 5. Comparative visualization of body shape differences between Bogor and Solok populations, illustrated in wireframe graph (A) and deformation grid (B). The landmark configuration connected with the blue line represented the body shape of the Solok population, while those associated with the green line represented the Bogor population (5A). In the deformation grid (5B), the Bogor population is indicated by the lolly, while the rod's tip indicates the Solok population. The length and projection of the rods show the orientation of shape changes between the two populations.

The GM applied in this study has captured, quantified, and visualized body shape differences in geographically distant and isolated populations of *O. vittatus*. The method has displayed a result of shape differences and shows in detail the point or combination of landmark points that changed, along with their orientation (projection of change). The latter property is not possible to observe with the TM. In light of the results in the present study, the shape information obtained through the GM can also be acquired by the TM method by looking at and measuring the ratio of body width over the body length at homological landmarks. While it is conceptually reasonable, the limitations of TM in removing the non-shape effect remained. Additionally, it can't tell further which parts of the body have changed or the projection of those changes.

While body shape variation between the two populations has been revealed, information on the forces driving those changes requires more investigation. The present preliminary study focused on implementing GM to capture body shape variation in specific populations. Therefore, explanations regarding the possible driving factors are based on secondary information, namely literature reviews. The present discussion is proposed, based on relevant theories and empirical studies, to give ideas on the possible factors that have been working that resulted in the observed phenomena. The phenotypic divergence of geographically isolated populations may result from adaptive evolution, genetic drift, and phenotypic plasticity [17]. An experimental study with Atlantic cod (*Gadus morhua*) proved that fish body shape possessed genetic and environmental components. This was revealed by differences in phenotypic plasticity among different populations in response to similar environmental factors [18]. Another evidence of the genetic effect on body shape can be found in hybrid populations, which in most cases showed intermediate diagnostic characteristics, such as indicated in a study with *Odontesthes* (Teleostei: Atheriniformes) in the Andean region of Southern South America [19].

Many studies have reported that morphological divergences were associated with selective pressure exerted by the habitat where the populations lived. Intraspecific morphological variation in fish is typically related to sexual dimorphism or one of three typical environmental gradients: variation in predation intensity, water velocity, or feeding niche [20]. Concerning feeding niches, for instance, fish that feed on benthos tend to have a downturned mouth. In contrast, those who feed on the pelagic usually have a terminal mouth, and those who feed on the surface typically have an upturned mouth structure [20]. In connection to water velocity, morphological and functional divergence were observed in two neotropical species (*Bryconops caudomaculatus*, Characidae, and *Biotodoma wavrini*, Cichlidae) which lived in the river channels and the lagoon basins. The maximum body depth

of the channel population for the two species was located more anteriorly than that of lagoon populations [21].

A study with Tangayikan cichlid assemblages, which tried to identify the genetic effect and ecological variates, found that body shape is most strongly predicted by ecological factors such as feeding preferences and water depth. Phylogenetic inertia had little effect on overall body shape [22]. A study with stickleback showed evidence of the presence of a habitat-body shape relationship. A divergence in body shape was observed in Stickleback, originating from environments differing in water velocity and feed sources [23].

Concerning the divergent body shapes observed in the *O. vittatus* populations under the current study, any driving force promoting the body shape variation as previously discussed is possible. Geographic isolation separating both populations, where no gene flow occurred for at least 10 thousand years following the last glaciation period, reflects a situation of allopatric divergence, which may lead to speciation. Under geographic isolation, both populations might evolve independently [24] and develop phenotypes and traits that best fit their environments [25]. The differences in environmental conditions such as site elevation, temperature, water velocity, bottom substrate, and feed source availability will all likely contribute to the observed phenomena. For instance, a study with salmonid showed that flow velocity affected body shape differently in salmon and trout. Salmon developed more robust body depth in a fast-flowing environment, while trout developed more streamlined [26].

Another study with the Neotropical fish genus *Rhoadsia* (Teleostei: Characidae) [17] found that body shape was a function of elevation, with those living in lower elevation showing a declined body depth. Populations of *O. vittatus* in this study were derived from river populations differing in elevation. The Cipunegara River crossing the Sumedang district from which the Bogor population originated was located at 50-240m asl, lower than that of the Liki River (385m asl), the source of the Solok population. The differential elevation between these two sites may result in differences in related abiotic factors, such as temperature, oxygen concentration, turbidity, water velocity, sediment particle size, energy input sources, and nutrient levels that eventually exert selective pressure on the body shape.

The current phenomenon, in which the population originated from a higher elevation had a broader body shape than that of the lower elevation, seemed in line with that found in the Neotropical fish genus *Rhoadsia*, as previously mentioned [17]. However, due to the complexity of variables associated with elevation, further efforts are needed to disentangle the major factors contributing to the divergence in body shape between the geographically isolated populations. Additionally, further research focusing on genetic analyses using either mitochondrial or nuclear markers would be helpful to explain the observed phenomena.

4 Conclusion

The geometric morphometric method detected, captured, and visualized morphological variation in body shape between bony lip barb populations of Bogor and Solok, which are geographically distant. Morphologically, the lateral view of the body shape of the Solok population was slightly broader and shorter. The Solok population's body shape looked stubby compared to the Bogor population, which looked slender.

References

1. L. F. Marcus, *Proceedings of the Michigan morphometrics workshop*, **2**, 77-122 (1990)
2. P. J. Park, W. E. Aguirre, D. A. Spikes, and J. M. Miyazaki, *Proceedings of the Association for Biology Laboratory Education*, **34**, 361-371 (2013)

3. M. L. Zelditch, D. L. Swiderski, H. D. a. Sheets, and W. L. Fink, *Geometric morphometrics for biologists: a primer* (Elsevier Academic Press, Massachusetts, 2004)
4. M. Kottelat, Raffles Bull. Zool. **27**, 1-663 (2013)
5. H. Syandri, J. Fish.Aquac. **5**, 1 (2014)
6. L. R. Parenti and K. Lim, Raffles Bull. Zool. **13**, 175-208 (2005)
7. V. Hasan, M. S. Widodo, and D. G. Wiadnya, AACL Bioflux, **12**, 1 (2019)
8. H. Syandri and J. Azrita, Int. J. Fish. **2**, 4 (2015)
9. Iskandar, Z. Anna, I. Zidni, D. Hermawan, and F. Pratiwy, IOP Conference Series: Earth and Environmental Science, IOP Publishing, **521**, 1 (2020)
10. N. Poulet, Y. Reyjol, H. Collier, and S. Lek Aquat. Sci. **67**, 122-127 (2005)
11. M. L. D. Worsham, E. P. Julius, C. C. Nice, P. H. Diaz, and D. G. Huffman, Ecol. Evol. **7**, 23 (2017)
12. L. U. Hepp, R. Fornel, R. M. Restello, A. Trevisan, and S. Santos, J. Crustac. Biol, **32**, 4 (2012)
13. C. Fruciano, C. Tigano, and V. Ferrito, Ital. J. Zool., **78**, 3 (2011)
14. F. J. Rohlf, Hystrix, **26**, 1 (2015)
15. I. L. Dryden and K. V. Mardia, *Statistical shape analysis: with applications in R.* (John Wiley & Sons, New York, 2016)
16. C. P. Klingenberg, Mol. Ecol. Resour., **11**, 2 (2011)
17. G. Malato et al., PLOS ONE, **12**, 6 (2017)
18. J. Marcil, D. P. Swain, and J. A. Hutchings, Biol. J. Linn. Soc. **88**, 3 (2006)
19. C. Conte-Grand, J. Sommer, G. Ortí, and V. Cussac, Neotrop. Ichthyol., **13**, 137-150 (2015)
20. E. Mattson and M. C. Belk, Open Fish Sci. J., **6**, 1, 87-91 (2013)
21. R. B. Langerhans, C. A. Layman, A. K. Langerhans, and T. J. Dewitt, Biol. J. Linn. Soc., **80**, 4 (2013)
22. C. Clabaut, P. M. E. Bunje, W. Salzburger, and A. Meyer, Evol., **61**, 3 (2007)
23. W. E. Aguirre, Biol. J. Linn. Soc., **98**, 1 (2009)
24. H. D. Lin, K. C. Hsu, K. T. Shao, Y. C. Chang, J. P. Wang, C. J. Lin, and T. Y. Chiang, J. Fish Biol., **72**, 8 (2008)
25. K. Johannesson and C. Andre, Mol. Ecol., **15**, 8 (2006)
26. S. Pakkasmaa and J. Piironen, Evol. Ecol., **14**, 721-730 (2000)