Genetic Variation of Populations of *Scutellaria slametensis* and *S. discolor* (Lamiaceae) on Gunung Slamet, Jawa Tengah (Indonesia)

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Abstract

Genetic variation within and between populations of *Scutellaria slametensis* Sudarmono & B.J.Conn and *S. discolor* Colebr. on Gunung Slamet (Jawa Tengah, Indonesia) are evaluated by allozyme electrophoresis. Gels stained by 4 enzyme systems, namely, *Aspartate aminotransferase* (Aat), *Esterase* (Est), *Malate dehydrogenase* (Mdh) and *Peroxidase* (Per), were used to evaluate the number of polymorphic loci. The mean of total number of observed alleles per locus (A), mean of total number of effective alleles per locus (A_e), percentage of polymorphic loci (Pp%), and expected genetic heterozygosity (H_e) have been generated as parameters of genetic variation. The interpopulation genetic differentiation (F_{ST}) and estimated geographic distance between populations were used to evaluate the correlation between genetic differentiation and geographic effect. It was found that *S. slametensis* is genetically distinct from *S. discolor* (D = 1.4572). The mean genetic variation of *S. slametensis* (Pp = 75 %, A = 2.00, H_E = 0.450) is greater than that of *S. discolor* (Pp = 25 %, A = 1.25, H_E = 0.125). Almost all loci of the latter species are monomorphic and homozygotic, especially population 9 near Baturaden (Pp = 0%; H_E = 0; Allele frequencies all = 1). There is a moderately high degree of variation between populations of these two species (F_{ST} = 0.585, SE ± 0.092), whereas within-population variation is low (1–F_{ST} = 0.415). Both species are out-breeding (at subpopulation level: F_{IS} = -0.973, SE ± 0.015; and population level: F_{IT} = 0.180, SE ± 0.183), with low levels of gene flow within and between populations (N_m = 0.249, SE ± 0.065). The chromosome number of *S. slametensis* and *S. discolor* is 2n = 24.

Introduction

Scutellaria (Lamiaceae) is the largest genus of the family with about 360 species (Huang, 1994; Paton, 1990, 2004). The genus is widespread, subcosmopolitan, but poorly represented in moist tropical lowlands. There are currently four known species in Indonesia, namely, *S. discolor* Colebr., *S. indica* L., *S. javanica* Jungh. and *S. slametensis* Sudarmono & B.J.Conn (Backer and Backhuizen van den Brink Jr, 1965; Keng, 1978; Steenis van, 1972; Sudarmono and Conn, 2010). The Indonesian species are all members of subgenus *Scutellaria* sect. *Scutellaria* (Paton, 1990) and are informally classified by Paton into several 'species-groups:' '*S. discolor* species-group' (including *S. discolor* and probably *S. slametensis*); '*S. humilis* species-group' (including *S. javanica*); '*S. violacea* species-group' (including *S. indica*).

Allozymes have provided the most abundant source of information regarding genetic variation in natural populations (Hamrick and Godt, 1990). Genetic variation parameters such as amount of interbreeding between populations, allele heterozygosity, genetic diversity, genetic differentiation and the amount of gene flow can indicate the level of gene mutation, genetic drift, genetic 'bottle necks', and even the possible level of vulnerability of endangered populations. These parameters are equally useful for assessing genetic variation within and between populations of a species as they are between species (Hamrick and Godt, 1990). Maki (1972) and Luzuko *et al.* (2000) found that there was a significant correlation between low gene flow and geographic distance or isolation by distance. Wright (1943) was the first to describe the genetic process of isolation by distance that may operate when populations conform to a 'stepping stone' model, where gene flow occurs only between adjacent populations. He demonstrated that random genetic drift within localized populations, combined with limited migration among populations, can result in increased genetic differentiation with increasing spatial distance between populations.

Grant (1981) concluded that geographic isolation was an important factor that may result in allopatric speciation with reproductive isolation playing an important role in sympatric speciation. Both of these speciation phenomena might occur within populations of *Scutellaria* on Gunung Slamet. *Scutellaria slametensis* occurs at elevations of more than 1,000 metres, whereas *S. discolor* occurs at elevations of less than 800 m. Chromosome numbers were recorded and within-population allozyme variation was analyzed for both species.

Materials and methods

Sample collections

Samples of *Scutellaria slametensis* were collected from six populations on Gunung Slamet (Jawa Tengah, Indonesia, Fig. 1). These populations occur at different elevations. Populations 1 (elevation 1,980 m) and 3 (1,802 m) occurring on the southwestern slopes, along the Kaligua and Brebes route in the Perseroan Terbatas Perkebunan Nusantara (PTPN) IX National Tea Plantation (in Paron and Sokarata Blocks of the Protected Forest, respectively), Forest Holding Unity Division – Balai Kesatuan Pemangku Hutan (BKPH), with population 2 occurring closer to the summit of Gunung Slamet (at 2,002 m) (Table 1). Populations 4-6, from the southern part of Gunung Slamet, on the Baturaden climbing track occur between elevations of 1,390 and 2,215 m (Table 1), on the eastern and southeastern slopes. Samples of three populations of *S. discolor* were collected at elevations less than 800 m, from the Baturaden area of Gunung Slamet (Table 1: populations 7-9). Although Keng (1978) concluded that *S. discolor* occurred over a wide range of elevations, from 500-2,400 m, this species was not found above 800 m. on Gunung Slamet. Ten individual plants were sampled from each population. Collections of all populations of both species were cultivated at Kebun Raya Bogor (Indonesia) and dried herbarium vouchers lodged at Herbarium Bogoriense (BO), Herbarium of Kebun Raya Bogor (KRB) and National Herbarium of New South Wales (NSW).

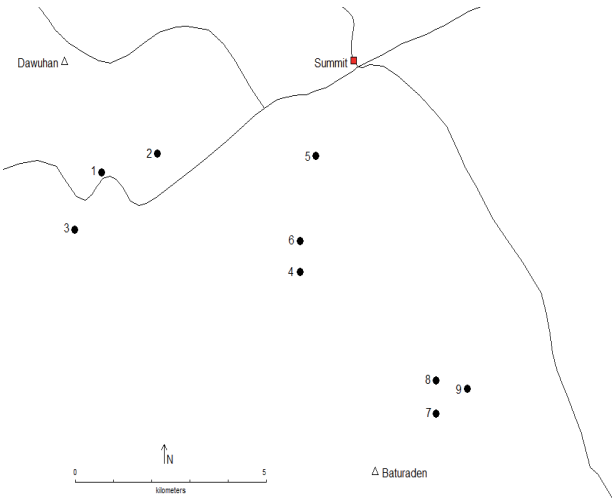

Figure 1. Distribution map of populations of *Scutellaria* included in this study on Gunung Slamet, Jawa Tengah, Indonesia. Populations 1-6 = *S. slametensis*; 7-9 = *S. discolor*; red square = summit of Gunung Slamet, elevation 3,428m.

Chromosome observation

Growing root tips were incubated in 0.05 % colchicine aqueous solution for 2 hours at 18°C. They were fixed with the fixative solution (ethanol: chloroform:glacial acetic acid = 2:1:1) for more than 45 minutes at 5°C. The root tips were then macerated with 1N HCl at 60°C for 18 seconds. The meristematic tissues were stained with 2% aceto-orcein for 5-10 minutes on a glass slide, one drop of 45% acetic acid was added and the tissue covered and squashed gently.

Allozyme analysis

Within-population allozyme variation was analyzed by enzyme systems for six populations of *Scutellaria slametensis* and compared to the within-population variation found within three populations of *S. discolor*, both occurring on Gunung Slamet (Jawa Tengah, Indonesia, Figure 1).

Leaf samples were prepared for allozyme electrophoresis by extracting 0.5 cm^2 samples of fresh leaf material. Young leaves were ground with 0.1 M Tris-HCl pH 7.5, 0.1% 2-mercaptoethanol, 0.001 M EDTA (tetrasodium salt). Extract was absorbed by filter paper (Whatmann No. 3) and run by 12% starch gel (4.5 hours, 300 volt; 45 mA). Four horizontal enzyme systems analyzed were Aspartate aminotransferase (*Aat*; EC 2.6.1.1), Esterase (*Est*; EC 3.1.1), Malate dehydrogenase (*Mdh*; EC 1.1.1.37) and Peroxidase (*Per*; EC 1.11.1.7). Staining procedure followed Soltis *et al*. (1983), with some modification in buffer pH and concentration (*Mdh* buffer with 1.5 M TRIS-HCl pH 8.0). The locus specifying the most anodally migrating isozyme was designated as 1, the next 2, and so on. Similarly, the most anodally of a gene was labeled 'a', the next 'b' and so on, as done by Shield *et al.* (1983) and Kephart (1990).

The genetic variation of populations is expressed as a percentage of polymorphic loci (*Pp* %), mean number of observed alleles per locus (*A*), and Nei's unbiased genetic diversity or expected heterozygocity (H_E), using POPGENE version 1.32 (Yeh *et al.*, 1999). Gene flow (N_m – the number of migrations per generation) for all loci was also estimated. The analysis of genetic identities (*I*) and genetic distance (*D*) for each pair-wise combination of populations were also estimated following Nei (1978). In this study, unbiased genetic identity was used to accommodate for the bias caused by small sample size (<50 individuals).

There are several indices used to evaluate genetic diversity, including total genetic diversity (H_T), genetic diversity within populations (H_S), genetic diversity among populations (D_{ST}), and the among populations genetic differentiation coefficient (G_{ST}). These statistics were generated using FSTAT program (Goudet, 2002). Total genetic diversity (H_T) was partitioned into within-population (H_S) and between-population (D_{ST}) components. H_T was estimated for each polymorphic locus from following

equations:

$$H_T = H_S + D_{ST}$$

$$H_S = \frac{1 - (\Sigma j_i)}{S}$$

Where, j_i is gene identity in sub-population and S is number of sub-populations. j_i was estimated by:

$$j_i = \Sigma x_{ik}^2$$

Where, x_{ik} is the frequency of the kth allele in ith sub-population

Genetic diversity between sub-populations (D_{ST}) was estimated by:

$$D_{ST} = (\Sigma_l \Sigma j \, D_{ij}) \, s^2$$

$$D_{ij} = \sum \frac{1}{2} (P_{ik} - P_{jk})^2$$

Where, P_{ik} is the frequency of the kth allele in ith sub-population, while P_{jk} is frequency of kth allele in jth sub-population.

Gene differentiation that occurs among populations (G_{ST}) was examined with Nei's genetic diversity indices (Nei, 1977, 1986). G_{ST} was expressed relative to total genetic variations among populations as:

$$G_{ST} = \frac{D_{ST}}{H_T}$$

Mean values of H_T, H_S, D_{ST} and G_{ST} were the average of all polymorphic loci within each group. All calculations were generated for each pair-wise comparison of populations and species using FSTAT program (Goudet, 2002).

The genetic structure of the studied populations was also analyzed in term of the following *F*-statistics: F_{IS} - fixation index related to non-random mating within populations; F_{IT} - mean inbreeding coefficient, F_{ST} - interpopulation genetic differentiation (following Weir and Cockerham, 1984).

Gene flow (N_m) for all loci was estimated:

$$N_m = \frac{(1 - G_{ST})}{4G_{ST}}$$

Assuming that populations have reached equilibrium between the effects of migration and random genetic drift, the degree of population subdivision was quantified using:

$$F_{ST} = \frac{1}{(4N_m + 1)}.$$

FST is the proportion of total genetic variance contained in subpopulation *S* relative to total genetic variance *T*. Unbiased data matrices (Nei, 1986) were generated using POPGENE (Yeh *et al*. 1999). It was also used to calculate mantel test (Sokal and Rohlf, 1995) for testing the null hypothesis of independence between genetic differentiation and geographic distances separating populations (Yeh *et al.*, 1999). Allozyme data were analyzed using UPGMA (Unweighted Pair-Group Method using Arithmetic Average) clustering techniques to construct a dendrogram to assist in the interpretation of these genetic data between species and populations. The dendrograms were generated using NTSYS (Rohlf, 2000).

Results

Chromosome analysis

Chromosome number of *Scutellaria slametensis* (Figs. 2A & B) and *S. discolor* (Figs. 2C & D) are both diploid 2n = 24. The length of mitotic metaphase chromosome of *S. slametensis* varies from 2-2.5 μm, whereas those of *S. discolor* are smaller (1.5-2 μm).

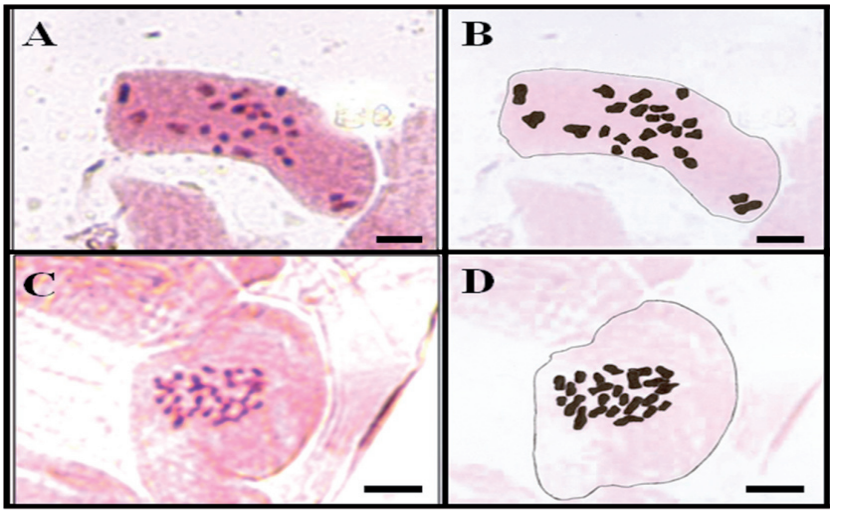

Figure 2. Mitotic metaphase chromosomes of *Scutellaria slametensis* (A, B) and *S. discolor* (C, D), both 2n=24. A & C, microphotographs; B & D, line-drawings of microphotographs. Scale bar = 5 μm.

Genetic Variation

Four loci were detected in all populations of both species of *Scutellaria* (Table 1). The four enzyme systems examined (namely, *Aat*, *Est*, *Mdh* and *Per*) were consistent for all nine populations studied. Populations 1, 4-6 (Table 1) are genetically highly variable with 75% of loci polymorphic (*Pp*). Population 4 has the highest mean number of alleles per locus (*A* = 2.00), with a high mean number of *A*=1.75 for populations 1, 5, 6 (Table 1). The expected heterozygosity is also regarded as high for these four populations (population 1: H_E = 0.375; 4: H_E = 0.401; 5: H_E = 0.375; 6: H_E = 0.375) (Table 1). In contrast, the lowest genetic variation occurs in population 9 (*Pp*= 0 %, *A* = 1.00, H_E = 0.000) (Table 1). Others populations that are regarded as having low genetic variation include: population 2 (*Pp* = 25 %, *A*=1.25, H_E = 0.139) and population 8 (*Pp* = 25 %, *A* = 1.25, H_E = 0.125).

Table 1. Mean genetic variation of populations of *Scutellaria slametensis* and *S. discolor* on Gunung Slamet (Jawa Tengah, Indonesia). Ten samples of each population (*N*) were examined. Populations 1-6 = *Scutellaria slametensis*; 7-9 = *S. discolor*. Percentage polymorphic loci (*Pp*), mean number of observed alleles per locus (*A*), mean of effective alleles per locus (*Ae*), mean of expected heterozygosity (H_E) (Nei, 1978). *Comparison of genetic variation categorized at regional geographic distributed means *Pp*=36.4 %, *A*=1.55, *Ae* =1.16 and H_E = 0.118 (Hamrick and Godt, 1990).

Pop. no.	Location	Altitude (m)	Geocode	*Pp%*	*A*	*Ae*	H_E	Category *
1	Protected forest block, Paron, Kaliwadas	1,980	7.2683° S 109.1584° E	75	1.75	1.75	0.375	high
2	Protected forest block, Sakub, Kaligua	2,002	7.2677° S 109.1456° E	25	1.25	1.25	0.139	Low
3	Protected forest block, Sokarata, Kaligua	1,802	7. 2829° S 109.1298° E	50	1.50	1.50	0.250	Low
4	Protected forest, Petak II	1,390	7.2834° S 109.2004° E	75	2.00	1.88	0.401	high
5	Protected forest, Post III, Baturaden	2,215	7.2668° S 109.2011° E	75	1.75	1.75	0.375	high
6	Protected forest, Post I, Baturaden	1,778	7.2668° S 109.2023° E	75	1.75	1.75	0.375	high
7	Camping ground, Baturaden	771	7.3119° S 109.2351° E	50	1.50	1.50	0.250	Low
8	Baturaden Botanic Garden	771	7.3052° S 109.2333° E	25	1.25	1.25	0.125	Low
9	Telaga Sunyi, Baturaden	777	7.3069° S 109.2427° E	0	1.00	1.00	0.000	low

Population structure and gene flow

Genetic diversity indices (Table 2) demonstrate that *Aat-1* and *Mdh* contain the highest total diversity (H_T) of those loci surveyed with 62.1% and 64.8%, respectively. The enzyme *Est*-1 contained the least genetic diversity (H_T = 44.4%). The mean of total genetic diversity (H_T) was 58% (SE ±0.030), whereas genetic diversity within populations (H_S) was 26.7% (SE ±0.058) and among populations (D_{ST}) 31.3% (SE ±0.034). There was no genetic diversity within-populations in the *Est-1* locus (H_S = 0%). Likewise, the genetic diversity within and among populations of *Aat-1* was low (H_S = 29.1% and D_{ST} = 35.1%, respectively). Although the genetic diversity within-populations of *Mdh* and *Per* were similar (both H_S = 38.9%), the among population genetic diversity was D_{ST} = 25.9% and D_{ST} = 19.8%, respectively. The mean genetic differentiation between populations (G_{ST}) is 57.1% (SE ± 0.095).

Table 2. Genetic diversity indices, *F*-statistic and estimation of gene flow between populations of *Scutellaria*. H_S, the genetic diversity within populations; D_{ST}, the genetic diversity among populations; H_T, the total genetic diversity; G_{ST}, the among populations gene differentiation coefficient; F_{IS}, the fixation index related to non random mating within populations; F_{IT}, the mean inbreeding coefficient of a set of a populations; F_{ST}, the interpopulation genetic differentiation; N_m, gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$. SE, standard error.

Locus	Genetic diversity indices				*F*-statistic			Gene flow
	H_S	D_{ST}	H_T	G_{ST}	F_{IS}	F_{IT}	F_{ST}	N_m
Aat-1	0.291	0.351	0.641	0.547	-0.919	0.135	0.549	0.205
Est-1	0.000	0.444	0.444	1.000	****	1.000	1.000	0.000
Mdh	0.389	0.259	0.648	0.400	-1.000	-0.091	0.455	0.300
Per	0.389	0.198	0.587	0.337	-1.000	-0.326	0.337	0.492
Mean	0.267	0.313	0.580	0.571	-0.973	0.180	0.585	0.249
SE	0.058	0.034	0.030	0.095	0.015	0.183	0.092	0.065

The estimation of the genetic variation among and within populations indicates a moderately high degree of differentiation among populations (F_{ST} = 0.585; SE ±0.092), whereas variation within-populations (1-F_{ST}) represented 41.5% of the total variance. These species exhibit a considerable degree of out-breeding at both the subpopulation (F_{IS} = -0.973; SE ±0.015) and population levels (F_{IT} = 0.180; SE ±0.183), even though the estimate of mean gene flow was low (N_m = 0.249; SE ±0.065). However, the null hypothesis that these populations are at equilibrium is rejected because there is no correlation between pair-wise genetic differentiation values (F_{ST}) and geographic distance (r = 0.365; Mantel *t*-test= 2.259; $p < 0.01$) (Fig. 4).

Based on genetic distance (Nei 1978), two population-groups are differentiated (Fig. 3), representing *Scutellaria discolor* (populations 7-9) which is genetically distinct from all populations of *S. slametensis* (Genetic distance, *D*=1.4572). Within the populations of the latter species, populations 1 (Paron block, Brebes) and 5 (population in Post III, along walking track from base camp Baturaden to summit of G. Slamet) are genetically similar (Fig. 3). Likewise, populations 4 (Post II) and 6 (Post I) are also genetically similar and this latter population pair is genetically close to the previous pair. Populations 8 and 9 (*S. discolor*) are also genetically similar (Fig. 3).

Allele frequency of 9 populations and allele shared among species

Most alleles are relatively common within the sampled populations (occurring in at least 50% of loci) (Table 3). However, two alleles with low frequencies in population 4 are *Aat-1*^{c} with only 15% of chromosomes carry this allele and *Aat-1*^{b} (35%). Alleles *Aat-1*^{c}, *Mdh-1*^{a,b,c} and *Per*^{b} were shared between *S. slametensis* and *S. discolor*, whereas, *Aat-1*$^{a, b}$, *Est-1*^{a} and *Per*^{a} were specific to *S. slametensis*. Alleles *Est-1*^{b} and *Per*^{c} are specific to *S. discolor*.

Table 3. Allele frequency of 4 polymorphic loci in 9 populations of *Scutellaria.* Populations 1-6 = *S. slametensis*; 7-9 = *S.discolor*

Population	*Aat-1*^{a}	*Aat-1*^{b}	*Aat-1*^{c}	*Est-1*^{a}	*Est-1*^{b}	*Mdh-1*^{a}	*Mdh-1*^{b}	*Mdh-1*^{c}	*Per*^{a}	*Per*^{b}	*Per*^{c}
1	0.50	0.50		1.00		0.50	0.50		0.50	0.50	
2	1.00			1.00			1.00		0.50	0.50	
3	0.50		0.50	1.00		0.50	0.50		1.00		
4	0.50	0.35	0.15			0.50		0.50	0.50	0.50	
5	0.50	0.50		1.00		0.50	0.50		0.50	0.50	
6	0.50	0.50		1.00		0.50		0.50	0.50	0.50	
7			1.00		1.00		0.50	0.50		0.50	0.50
8			1.00		1.00	1.00				0.50	0.50
9			1.00		1.00	1.00				1.00	

Discussion

Paton (2004) noted that the chromosome numbers for *Scutellaria* are 2n=12-88, with most frequent numbers being 2n=20, 22, 24, 32 and 34. Chromosome numbers of *Scutellaria* subgenus *Scutellaria*, which includes all Indonesian species, are 2n=24-34 (Paton, 1990). Since the chromosome numbers of *S. discolor* and *S. slametensis* are both 2n=24, these two species are possibly closely related. Both of these species have small chromosomes, less than 5 µm long.

Allozymes have been successfully used to compare mating system, migration and local differentiation within and between populations (Brown,

1990). Factors such as, regional distribution of a taxon, geographic range, breeding system, seed dispersal mechanisms and successional status have been associated with differences in the percentages of polymorphic loci (*Pp*%), mean number of observed alleles per locus (*A*), and genetic diversity within-populations (H_E) (Hamrick and Godt, 1990). Values of *Pp*%, *A* and H_E obtained for *S. discolor* and *S. slametensis* are very similar to those obtained for out-breeding plants, wide-spread species, and for those with seeds that are dispersed by gravitational forces.

Scutellaria slametensis is a species with a restricted distribution and has mean genetic variation (*Pp*= 75 %, *A* = 2.00, H_E = 0.450) equivalent to that of *S. montana* (*Pp* = 75.42 %, *A* = 2.21, H_E = 0.19; see Cruzan, 2001), a species restricted to parts of Georgia and Tennessee (U.S.A.) (Cruzan, 2001). *Scutellaria slametensis* exhibits greater genetic variation than plants of *S. discolor* sampled from G. Slamet. Almost all loci of the latter species are monomorphic and homozygotic, especially population 9 (*Pp* = 0 %; H_E = 0; Allele frequencies all=1).

The value of allozyme electrophoresis in delimiting taxa has been found to be useful by several researchers (such as, Coates and Hnatiuk, 1990; Crawford, 1985; Gottleib, 1984). Although all populations of *S. discolor* and *S. slametensis* are relatively close geographically, they are genetically distant based on allozyme divergence. This is possibly a result of the steep mountainous terrain restricting the potential pollinators to small geographical areas within the region (Tyler, 2003). Within *S. slametensis*, populations 4 and 6 are genetically and geographically close. Population 5 is unexpectedly genetically close to population 1 even though the two are not geographically close. Together, populations 1 and 5, and 4 and 6 form a genetically distinct grouping (Fig. 3). Although the two high altitude populations, 5 (elevation at 2,215 m) and 2 (2,002 m) are geographically relatively close, these two populations are genetically distant (Fig. 3). Although population 1 is located in between populations 2 and 3, the genetic variation, hence gene flow, within the latter two populations is low suggesting that they do not share a common population of pollinators. Contrary to this, within-population variation in the population group 1 and 4-6 is high, representing high gene flow between these populations (Table 1). The three populations of *S. discolor* (populations 7-9) are both geographically close and genetically similar. Species characterized by a low level of gene-flow, such as *S. discolor* (Table 1), or a low level within or between populations, such as populations 2 and 3 (*S. slametensis*), may indicate high levels of selfing. Cruzan (2001) hypothesized that the smaller fragmentation threshold may reflect higher levels of selfing in isolated populations because of the absence of pollinators. Unpublished results from observations of floral behaviour and crossing tests of *S. slametensis* clearly demonstrate that this species is mostly self-

compatible as has been found in *S. indica* (Sun, 1999). However, within the current study, the low overall proportion of total genetic variance within a subpopulation relative to total genetic variance (F_{ST} = -0.9769) implies a low level of differentiation among populations, suggesting that *S. discolor* and *S. slametensis* are out-breeding. The low level of gene flow (mean N_m = 0.1988) observed suggests that although these populations are geographically close, they are relatively isolated genetically. Slatkin (1987) concluded that values of $N_m < 1$ mean that genetic drift will result in substantial differentiation between populations. This is possibly a consequence of pollinators only visiting flowers within a restricted area, hence mostly visiting plants in closely adjacent areas (such as, between populations 4 and 6, and between 8 and 9). However, this does not explain the lack of genetic distance between populations 1 and 5 (D = 0.000).

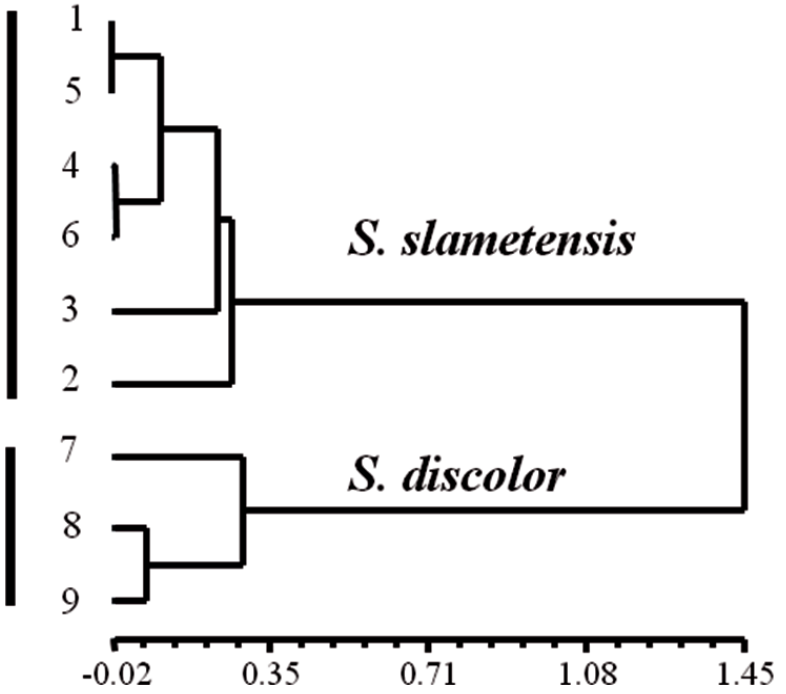

Figure 3. Dendrogram of Nei's genetic distance (Nei, 1978) between six populations of *Scutellaria slametensis* and three populations of *S. discolor* on Gunung Slamet, Jawa Tengah, Indonesia.

Mean total genetic diversity (H_T) showed majority of partitioning occurred among populations (D_{ST} = 31.5%) rather than within populations (H_S = 0.267). The large amount of genetic differentiation among populations

(G_{ST} = 0.571; F_{ST} = 0.585) indicate strong genetic differentiation between these populations (Table 3). *F*-statistics have similar values and indicate that populations of *S. discolor* and *S. slametensis* are genetically structured (Table 2). High levels of genetic structure within-populations are supported by the *Est-1* locus but with low levels of gene flow (*Nm*) (Table 2). High levels of pollen transfer indicate movement of pollinators that will naturally lead to increased genetic differentiation but decreased gene flow (Ellstrand and Elam, 1993). If genetic variation within species is predominantly affected by shared alleles, then there may be a significant correlation between shared alleles and the local geographical distribution pattern of the species. An understanding of nature of shared allele may be used to understand conservation implication for future.

Throughout this study we have assumed a 'stepping-stone' model (Kimura, 1953) of population structure among these plants whose dispersal ability is constrained by distance such that gene flow is most likely to occur between neighbouring populations (Hutchinson and Templeton, 1999). Consequently, it would be expected that adjacent populations tend to be more genetically similar than more distant populations. Therefore, assuming a 'stepping-stone' model of regional population structure, the null hypothesis that these regional populations are at equilibrium is rejected because there is no association between pair-wise genetic differentiation values (F_{ST}) and geographic distance (r = 0.365; Mantel *t*-test= 2.259; $p < 0.01$) (Fig. 4). In this study, the populations of *Scutellaria* on Gunung Slamet consist of mostly small, more or less isolated populations, such that allele frequency drifts independently of geographic distance between populations and is much more influential in determining the population structure than gene flow. These results are consistent with limited pollination events occurring between populations and/or the inhibition of dispersal in this mountainous terrain.

The dendrogram of the sampled populations (UPGMA – based on Nei's genetic distances; Nei, 1978) indicates a correlation between shared alleles and genetic distance (*D*) (Fig. 3). The topology of this dendrogram is congruent with morphological differences between *S. discolor* and *S. slametensis*. Based on allozyme data, the greatest genetic distance (*D* =1.45) was found between *S. discolor* and *S. slametensis*. There are several morphological features that distinguish *S. discolor* and *S. slametensis* (Sudarmono and Conn, 2010). The shape of the leaf, height of stem and number of flowers at inflorescence nodes of *S. discolor* are very different from those of *S. slametensis*. *Scutellaria discolor* has flowers with corolla purple, whereas, *S. slametensis* has corollas that are white basally, pink distally (populations 1 & 2) or purple distally (populations 3-6). *Scutellaria slametensis* has obovate leaves, whereas those of *S. discolor* are ovate. The former species has two flowers at each node of the inflorescence, whereas *S. discolor* has four flowers per node.

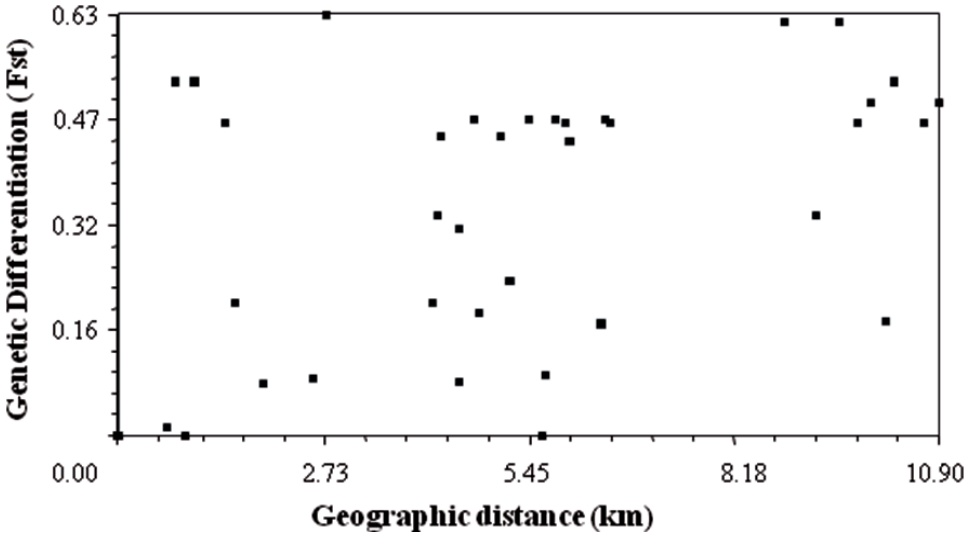

Figure 4. Correlation between geographic distance (km) and genetic differentiation (F_{ST}) of nine populations of *Scutellaria discolor* and *S. slametensis* on Gunung Slamet. r=0.365; Mantel *t* test = 2.259; $p < 0.01$.

Conclusion

Scutellaria discolor and *S. slametensis* are morphologically and genetically distinct. Genetic variation within populations 1, and 4-6 are high (*S. slametensis*), whereas within populations 2, 3 (both *S. slametensis*), and 7-9 (*S. discolor*) are low. Although the genetic diversity within *S. slametensis* is partitioned, with one group with high genetic variation (populations 1, 4-6) and one with low values (populations 2 and 3), overall this species has a high level of genetic heterozygosity that makes it a species with a high frequency of heterogamy. Consequently, from a conservation point of view, this species is probably a low-risk species (Fracaro and Echeverrigaray, 2006). Even though the two species at the subpopulation level (F_{IS} = -0.973; SE ±0.015) and population level (F_{IT} = 0.180; SE ±0.183) are out-breeding, the mean gene flow was low (N_m = 0.249; SE ±0.065). Historically, genetic drift has affected the overall structure of the populations of this species, rather than gene flow. However, within the distribution of *S. slametensis*, the mean gene flow was high for the local populations 1, 4-6. Since this species is only known by a limited number of individuals in a restricted locality, it may prove to be vulnerable (IUCN, 2001) should the populations become more isolated, resulting in the level of gene flow and hence, heterozygosity becoming further reduced.

Although *S. discolor* is a widespread species, all loci of the plants sampled on Gunung Slamet are monomorphic and homozygotic. An assessment of the genetic variability of this species throughout its range is required. Even though a species may be widespread, an increased homozygosity may result in a reduction of the vigor of individuals, expression of deleterious characters, increased seed abortion, reduced fertilization and germination rates. These are factors that may lead to the disappearance of the populations (Dubash and Fenster, 2000).

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