Genetic differentiation of 3 *Leptocarabus* beetles that inhabit different altitudinal zones in Japanese Alps

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Background: >half of species of carabid beetles (Coleoptera; Carabina) speciated in Japan. The most famous hypothesis is the allopatric speciation.

Question: *In carabid beetles, were gene flow restricted by geographic topology?*

① Genetic differentiation between mountains?

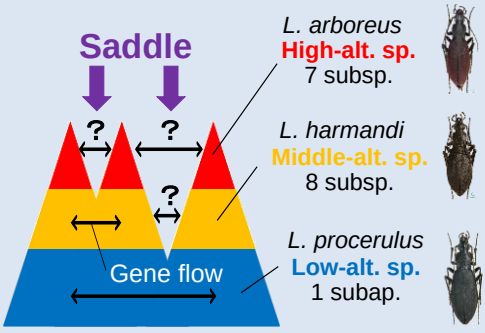


Fig. 1. In central Japan, 3 *Leptocarabus* beetles the inhabit different altitude zones. Their subspecies were recorded by only morphology.

② Sampling sites; the central Japan

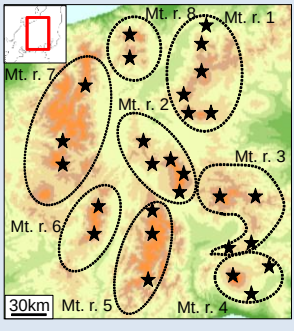


Fig. 2. We set >2 points (black stars) in each mountain regions (Mt., dotted line). Total 28 points.

Table 1. Result of sampling. We used 1-2 individuals in each point in each species for DNA analysis.

	Low-alt. sp.	Middle-alt. sp.	High-alt. sp.
Points	27	7	22
Subspecies	1	5	7
Individuals	269	15	208
Analysis	36	8	40

③ High-alt. sp. has the most number of haplotypes (28s rDNA).

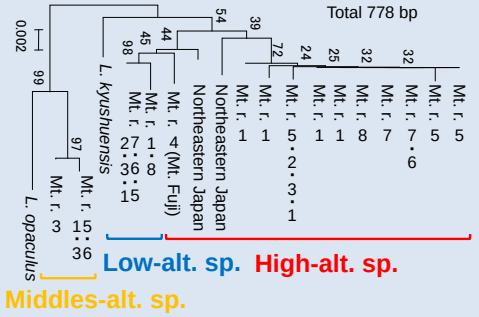


Fig. 3. Phylogenetic tree of 28s rDNA used by neighbor-joining method. We added samples from northeastern Japan and outgroups (*L. opaculus, L. kyushuensis*) which were supplied by Prof. Sota (Univ. Kyoto) and Mr. Narita (Aizu high school).

④ In High-alt. sp., the same haplotypes are shared between high altitude (>1001m) saddles.

Some subspecies are not discriminated by 28s haplotype.

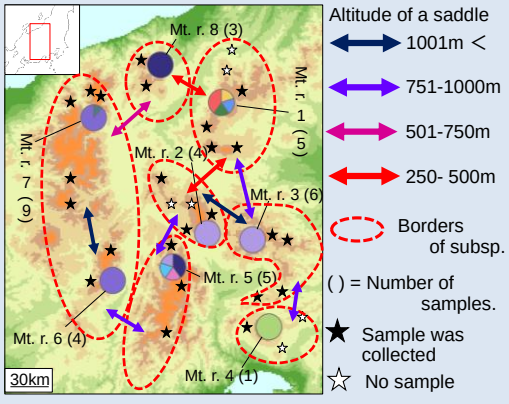
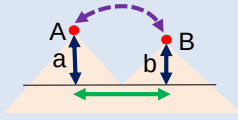


Fig. 4. Haplotypes distributions of 28s rDNA of High-alt. sp..

⑤ Saddles promoted genetic dist. in only High-alt. sp..

Genetic dist. ～ **Horiz. dist.**＋**Alt. dist.**
(Multiple regression, All pairwise between points, Mantel-like *P* correction)



Genetic dist.＝ **28s (778bp)+Wg (469bp)**
Alt. dist.＝**a + b**

⑤-1 Genetic dist. vs Horiz. dist.

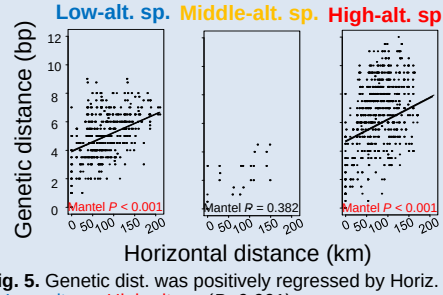


Fig. 5. Genetic dist. was positively regressed by Horiz. dist. in Low-alt. sp, High-alt. sp (*P<0.001*).

⑤-2 Genetic dist. vs Alt. dist.

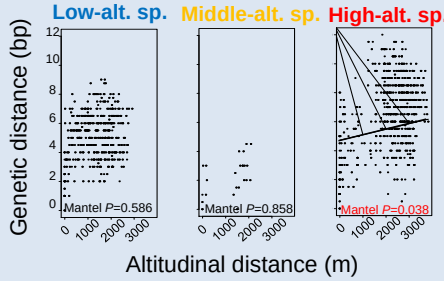


Fig. 6. Genetic dist. was positively regressed by Alt. dist. only in High-alt. sp (*P<0.05*).

Conclusion: Low-altitude saddles make geographic barriers for gene flow in High-alt. sp..

We will examine the relationship between subspecies and genetic differentiations using more genes and samples.