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Maintenance mechanism of a supergene for shell colour polymorphism in the terrestrial pulmonate *Bradybaena similaris*

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The land snail *Bradybaena similaris* exhibits polymorphism for the ground colour of periostracum and the banding pattern of ostracum and periostracum. The ground colour is either dark reddish brown or pale light brown. In addition, a single chestnut-color band is either present or absent along the ridge of the whorl. Thus, there are four morphs in combination: dark and banded, dark and unbanded, light and banded, and light and unbanded. Earlier breeding experiments have shown that the phenotypes of ground colour and banding are controlled by two linked loci, where the *dark* (C^+) and *banded* (B^+) alleles are dominant to the *light* (C^-) and *unbanded* (B^-) alleles. Thus, four different *cis* configurations of alleles (C^+B^+, C^+B^-, C^-B^+ and C^-B^-) could be expected. However, the C^+B^+ chromosome has not been found, i.e. all the examined specimens of the dark/banded morph were *trans* heterozygotes (C^+B^-/C^-B^+). This linkage disequilibrium may result from tight linkage, lethality of the dark/banded haplotype or recombination suppression. We found no recombinant phenotype in 3287 progeny obtained by the test cross of C^+B^-/C^-B^+ with C^-B^-/C^-B^- in 16 pairs. Our results suggest that recombination between the colour and banding loci is suppressed in effect.

Key words: Gastropoda, Pulmonata, Bradybaenidae, *Bradybaena similaris*, colour pattern, linkage disequilibrium, dominance.

INTRODUCTION

Shell colour polymorphism in land snails has been studied to understand the causes of visual genetic variation in natural populations (Jones et al., 1977; Clarke, 1978; Clarke et al., 1978; Cain, 1983; Cowie, 1992; Cook, 1998; Honek, 2003; Hayashi & Chiba, 2004). The shell polymorphism of *Cepaea nemoralis* (L.) is one of the earliest examples of genetic variation in animals by which Mendelian segregation was confirmed (Lang, 1904). Since then, detailed knowledge of inheritance has been obtained for a few species of land snails (Murray, 1975; Clarke et al., 1978). The genetic basis of shell colour morphs in *C. nemoralis* has made it possible to disclose the roles of natural selection in maintenance of colour polymorphism and in divergence of colour patterns between populations (Jones et al., 1977; Cain, 1983; Cook, 1998, 2005, 2007; Cook et al., 1999; Davison & Clarke, 2000; Ozgo, 2005).

Molluscs in general, however, are not easy material for genetic study, because most molluscs are difficult to rear, and the generation time is longer than a few years in many terrestrial gastropods. For those reasons, the genetic basis of shell polymorphism is little understood in general, even in the Helicidae and Bradybaenidae which are known for shell colour polymorphism in many species. In contrast, *Bradybaena similaris* (Rang, 1831) has several advantages for genetic studies of polymorphism for the ground colour and banding of the shell in natural populations. First, the generation time is relatively short (3 months in the laboratory), compared with *Cepaea* (2 years). Second, the body size is small (12 mm shell diameter). Third, populations are usually dense and it is easy to obtain large

samples from small areas. Fourth, the genetic basis of the shell colour polymorphism is relatively simple. Fifth, a protocol for laboratory breeding has been established (Asami & Ohbayashi, 1999).

In natural populations of *B. similaris*, four shell morphs are found in combination of ground colour and banding patterns; dark brown and banded (DB), dark brown and unbanded (DU), light brown and banded (LB), light brown and unbanded (LU). Since Komai & Emura (1955), DB, DU, LB and LU have been called BS (brown and striped), BU (brown and unstriped), YS (yellow and striped) and YU (yellow and unstriped), respectively. However, the striped phenotype, which S indicates, has often been called banded and indicated by B (Murray, 1975), and thus the use of B to indicate the brown is confusing. YS (yellow and striped) and YU (yellow and unstriped) are also confusing, because those morphs do not exhibit yellow pigmentation, while the four morphs of ground colour and banding patterns are similarly found in a sibling species, *B. pellucida* Kuroda & Habe, the dorsal mantle of which shows bright yellow through the shell (Asami et al., 1997a,b). Thus, here we use DB, DU, LB and LU, instead of BS, BU, YS and YU.

In the Bradybaenidae, the maximum number of bands that may appear on the shell is four, while that in the Helicidae is five. In a conventional way to describe the banding pattern, the banded morphs of *B. similaris* correspond to 0200 in the banding system of the Bradybaenidae. The shell colour morphs are determined at two putative loci, *C* for the ground colour of the periostracum and *B* for the banding of both periostracum and ostracum. According to Komai & Emura (1955), the *dark-brown* allele (C^+) is dominant to the *light-brown* allele (C^-), and the *banded* allele (B^+) dominant to the *unbanded* allele (B^-). These two loci show strong linkage in inheritance. In this genetic model, there are four possible chromosomal configurations; C^+B^+, C^+B^-, C^-B^+ and C^-B^-. Because of complete dominance of alleles at both loci, genotypes of individuals other than the light-brown and unbanded morphs can only be determined by crossing experiments. Throughout examination of individual genotypes, the chromosome of *cis* configuration C^+B^+ has not been found, and all the individuals of DB were *trans* heterozygotes with C^+B^- and C^-B^+ (Ikeda & Emura, 1937; Komai & Emura, 1955). Thus, the inheritance of the ground colour and banding patterns could also be explained by assuming three alleles (*DU*, *LB* and *LU*) at a single locus for both ground colour and banding (Ikeda & Emura, 1937). It is, however, unlikely that the ground pigmentation of periostracum and the banding of ostracum and periostracum are controlled by the same gene, according to the genetic systems for shell colour polymorphism in other pulmonates (Murray, 1975; Clarke et al., 1978). Instead, the absence of the C^+B^+ chromosome should indicate the linkage disequilibrium of genotypic frequencies at the two loci in natural populations.

In this study, we tested the mutually exclusive predictions of three hypotheses of a mechanism that causes the linkage disequilibrium at the colour and banding loci in *B. similaris* by the test cross of the *trans* heterozygotes DB (C^+B^-/C^-B^+) with the double recessive homozygotes LU (C^-B^-/C^-B^-). Our results suggest that the linkage disequilibrium is maintained by suppression of recombination between the two loci.

MATERIALS AND METHODS

Because of general low frequencies of DB in Japanese populations of *B. similaris* (Komai & Emura, 1955; Asami & Ohba, 1982), it is not practical to attempt to collect juvenile DB enough to replicate the test cross of DB with LU. Because both C^+ and B^+ are generally rare alleles in the wild, DU and LB are mostly heterozygous at the *C* and *B* loci, respectively and produce DB by crossing with each other. Thus, we collected 10 DU juve-

niles and 10 LB juveniles from a small area (10 × 1 m; 35° 37′ 23″ N, 139° 33′ 10″ E) in Ikuta, Tokyo. Snails from such a small area should be a sample of a single population, which is not subject to chromosomal heterosis or hybrid breakdown. For all the present procedures to prepare virgin adults to cross and to obtain their offspring, we employed the protocols of Asami & Ohbayashi (1999), except for the temperature (20°C) and using sand as the oviposition substrate.

We raised DU and LB juveniles to maturation individually in plastic containers (62 × 50 × 25 mm). After their maturation, we kept each of four pairs of DU and LB in a flowerpot with the oviposition substrate to let them reproduce. We obtained DB and LU offspring from those parents and individually raised them to maturation. We pursued the test cross by 16 pairs of DB and LU that we obtained from different pairs of parents to avoid possible inbreeding depression. We first kept each pair in a plastic container (109 × 79 × 32 mm) for mating for three weeks. Then we separated each pair into two flowerpots with the oviposition substrate, from which we collected eggs once a week until they died. Similarly to *B. pellucida* (Asami et al., 1993), the banding phenotype of juveniles can be exactly identified within two weeks after hatching, when they are reared in the laboratory. However, the dark brown phenotype may not be dark enough to distinguish from the light brown phenotype earlier than one month after hatching (Asami et al., 1997a). Thus, we determined the ground colour and banding phenotypes of the progeny later than one month after hatching.

HYPOTHESES

The test cross of DB with LU can test three mutually exclusive hypotheses of the mechanism of the linkage disequilibrium. The following hypotheses commonly assume that the ground colour and banding of the shell are controlled by different loci C and B, respectively, and that the *cis* configuration of the C^+ and B^+ alleles is significantly less frequent than expected from the random combination of the frequencies of those alleles in natural populations.

Linkage hypothesis. – The C and B loci are located at close positions on a chromosome, and the rate of recombination of these loci is low. *B. similaris* has been introduced into Japan perhaps only a few hundred years ago. The generation time is about one year in the wild. Populations may be repeating colonization and extinction in ephemeral habitats in artificially disturbed areas. Thus, most populations in suburbs would not have reached linkage equilibrium. This hypothesis predicts that recombination occurs at a low frequency and that recombinants can be detected by the test cross with a large number of replicates.

Suppression hypothesis. – Recombination between the two loci is suppressed by a certain mechanism. For example, inversion polymorphism effectively suppresses recombination through the lethality of the recombinant chromosome carrying the C^+ and B^+ alleles and that carrying the C^- and B^- alleles, if the C and B loci are located within a chromosomal section that is polymorphic for inversion and the C^+B^- and C^-B^+ chromosomes differ from each other in the arrangement of the inverted section. Prediction is that the test cross produces no recombinant no matter how large the replication size would be.

Lethality hypothesis. – The *cis* configuration of C^+ and B^+ causes a lethal effect on the chromosome. This hypothesis predicts that the recombinant with the phenotype LU appears in the test cross, while no recombinant with C^+ and B^+ in the *cis* configuration can be obtained.

Testing hypotheses

B. similaris is hermaphroditic but seldom reproduces by self-fertilization (Ueshima & Asami, 2003). However, it has been shown that the progeny by self-fertilization could be included in clutches laid by mated animals (Komai & Emura 1955). Thus, the test cross for the present purpose needs to be designed so as to be able to distinguish recombinants from the progeny produced by self-fertilization.

If crossing over occurs between the *C* and *B* loci in the parental DB, and the recombinants are viable, the recombinant phenotypes DB and LU should appear in progeny of both the parental DB and LU. On the other hand, self-fertilization by the parental LU produces LU, and that by the parental DB could produce three phenotypes, DB, DU, and LB. Accordingly, the recombinant phenotypes that can be distinguished from the phenotypes of self-fertilized progeny are DB from the parental LU, and LU from the parental DB, in the present test cross.

In addition, the DB offspring produced by the DB parent can be crossed with LU to test whether it is a recombinant. That is, if it carries the *cis* configuration of C^+ and B^+ as a recombinant, the progeny of this test cross should show segregation of DB and LU in 1:1. If not, DU and LB would segregate in 1:1. If the LU offspring are obtained from the DB parent, and no DB offspring from the LU parent, this result supports the lethality hypothesis. However, to accept this hypothesis, this pattern of result should be confirmed by repeated production of LU without DB.

RESULTS

A total of 3287 progeny were phenotyped for the ground color and banding (table 1). Every parent produced both the DU and LB offspring. The 6 DB offspring were all obtained from the DB parents, from which no LU progeny was obtained. The 5 LU offspring were all from the LU parents, from which no DB progeny was obtained. Thus, these DB and LU offspring can be ascribed to selfing by their parents. The segregation ratio between the DU and LB offspring did not significantly differ from 1:1 (χ^2 test, P = 0.38, assuming no interaction between the mating partners). Therefore, the present results support the suppression hypothesis.

The mean number of eggs laid by the parents of the DB and LU progeny was 50.0, and their mean survival rate till each progeny was phenotyped about one month after hatching was 0.543, whereas for the other parents, the number of eggs was 127 and the survival rate was 0.891 in average (Table 1). Their differences were statistically significant (Mann-Whitney test, P = 0.0003 for the number of eggs, P = 0.0008 for survival). These results suggest that the parents of the DB and LU offspring reproduced less successfully because of inbreeding depression resulting from selfing. There were no significant differences between the DB and LU parents in the number of eggs (P = 0.78) or in the hatchlings' survival (P = 0.69).

DISCUSSION

The rarity of the DB chromosome in natural populations of *B. similaris* is a clear indication of linkage disequilibrium unless the ground colour and banding are controlled by a single locus. The present results suggest that recombination between the colour and banding loci is effectively suppressed and thus the linkage disequilibrium is likely maintained stably. Logically the absence of recombination cannot be demonstrated, i.e. an

Pair no.	Parent	no. eggs	no. days	DU	LB	DB	LU	Total	Survival
1	DB	70	160	33	30	0	0	63	0.900
	LU	101	190	42	39	0	0	81	0.802
2	DB	148	280	75	66	0	0	141	0.953
	LU	250	182	116	105	0	0	221	0.884
3	DB	121	147	49	40	0	0	89	0.736
	LU	95	133	45	40	0	0	85	0.895
4	DB	166	271	71	79	0	0	150	0.904
	LU	110	220	60	42	0	0	102	0.927
5	DB	100	162	53	43	0	0	96	0.960
	LU	41	123	14	10	0	2	26	0.634
6	DB	109	220	41	35	0	0	76	0.697
	LU	156	171	70	66	0	0	136	0.872
7	DB	155	181	61	88	0	0	149	0.961
	LU	118	184	45	61	0	0	106	0.898
8	DB	106	251	55	40	0	0	95	0.896
	LU	36	230	6	2	0	3	11	0.306
9	DB	94	150	42	48	0	0	90	0.957
	LU	137	154	59	68	0	0	127	0.927
10	DB	150	222	71	57	0	0	128	0.853
	LU	98	76	43	39	0	0	82	0.837
11	DB	120	181	59	44	0	0	103	0.858
	LU	41	40	19	17	0	0	36	0.878
12	DB	86	157	36	40	0	0	76	0.884
	LU	155	250	62	82	0	0	144	0.929
13	DB	160	180	69	83	0	0	152	0.950
	LU	173	198	85	69	0	0	154	0.890
14	DB	67	123	24	19	3	0	46	0.687
	LU	126	189	55	62	0	0	117	0.929
15	DB	57	24	13	15	3	0	31	0.544
	LU	141	148	63	71	0	0	134	0.950
16	DB	118	160	50	57	0	0	107	0.907
	LU	144	197	77	56	0	0	133	0.924
Total		3749	-	1663	1613	6	5	3287	-
Mean		117.2	174	-	-	-	-	102.7	0.848

Table **1**. The results of test cross between DB and LU in *Bradybaena similaris* from the initial to the last ovoposition.

empirical study cannot deny that recombination between the two loci might be possible but too rare to detect. In practice, however, even if one putative recombinant were found among, for example, ten times as much progeny as obtained in this study, it could no longer be distinguishable from a mutant at either one of the two loci. By the present scale of the test cross, our results show the absence of experimentally detectable recombination between the two loci in *B. similaris*.

Breeding experiments by Komai & Emura (1955) included the test cross of DB with LU by 9 pairs and the cross of DB with DB by 178 pairs. In that test cross, the 9 LU parents produced 12 LU young but no DB in total of 855 offspring. The 9 other DB parents produced no LU but 1 DB young in total of 851 offspring. These LU and DB offspring can

be attributed to self-fertilization, because no clear recombinants of DB and LU were obtained from the LU and DB parents, respectively. On the other hand, in the cross of DB with DB, 3 LU individuals were produced in total of 6596 offspring. This pattern of phenotypic segregation might suggest crossing over between the C and S loci, producing the *cis* configuration of C^- and B^- from chromosomes of the *trans* heterozygotes. However, both parents were DB, and so the LU progeny requires crossovers in both the parents, or selfing after crossovers in both spermatogenesis and oogenesis in either one of the parents. Thus, if the LU offspring resulted from recombination, the recombination rate would be 0.021. This value is apparently too high to explain linkage disequilibrium observed in natural populations and contradicts the absence of definite recombinants in the other crosses by Komai & Emura (1955) and in the present results. Thus, those 3 LU offspring must have resulted from some accidental artifact.

It has been shown in some species of land snails that polymorphic characters such as the ground colour, banding and the lip colour are controlled by a genetic system with closely linked loci, a supergene (Cain et al., 1960, 1968; Murray & Clarke, 1976a, b). The shell polymorphism of *C. nemoralis* involves at least nine loci, and five of them show strong linkage disequilibrium, forming a supergene of functionally related alleles (Cook, 1998, 2005). Similarly, in *C. hortensis* (Müller), four loci for the ground color and banding show tight linkage in inheritance (Murray, 1963; Cook & Murray, 1966). In *Partula taeniata* Mörch, six loci for shell patterns have been identified and are all linked. The shells of *P. suturalis* Pfeiffer show complex patterns of colour and banding as well. It is notable that the shells of those species do not show close correspondence in polymorphic patterns which are controlled by supergenes with different systems, in spite of their close relatedness (Murray & Clarke, 1976a, b).

These examples including the current case of *B. similaris* indicate that tight linkage among loci for shell patterns has been established similarly in different lineages of evolution in pulmonates, i.e. the Helicidae, Partulidae, and Bradybaenidae. These linkage groups of loci for shell patterns in the three families, however, would not be evolutionarily homologous, but instead have resulted from convergence (Murray & Clarke, 1976a). The linked loci in the supergene often code the shell and lip colour and the banding which are functionally related in ecology and in physiology. It is apparent that the shells physically protect terrestrial molluscs from desiccation and predators, determining the entire appearance of their bodies retracted in their habitats. It has been shown that the colour and banding patterns of *C. nemoralis* strongly affect fitness because of their differential mortalities due to predation by thrushes (Cook, 1998). Shell colour also functions for thermal adaptation to a variety of physical conditions of the environment (Cain, 1983; Cook, 1998). Therefore, it is likely that these systems for linkage have been derived independently by means of natural selection for advantageous combinations of alleles. In *Arianta arbustorum*, the recombination rate of the shell colour loci has been estimated at 0.2% (Cook & King, 1966). In comparison, the present results clearly indicate that the genetic system of the supergene in *B. similaris* is different in terms of the absence of detectable recombination.

The generation time is two to three years in *Cepaea* and often longer than a year in *Partula* in the laboratory (Murray & Clarke 1966). Their genetic systems for shell polymorphism are complex. Those have been common problems for studies of the mechanisms of linkage disequilibrium. Because of ovoviviparity, the reproduction rate of *Partula* is relatively low compared with oviparous snails. In these groups, therefore, there have been few studies on recombination within the supergene and on the mechanism of tight linkage in inheritance. In comparison, *B. similaris* has advantages essential for the genetic study of a supergene; simplicity of the shell polymorphism, the short generation time, the

high rate of copulation and reproduction (Komai & Emura, 1955; Asami et al., 1998; Asami & Ohbayahsi, 1999). The present study has shown that these advantageous traits allow a simple design of breeding experiments to pursue critical tests of hypotheses on the mechanism of linkage in question.

Cook (2007) has found that the shell breadths of dominant phenotypes in ground colour and banding are larger and exhibit less variance than those of recessive homozygotes in *C. nemoralis*. Because individuals of the dominant phenotypes were largely heterozygotes, it shows that heterozygous advantage contributes to the maintenance of shell colour polymorphism, as Goodhart (1987) and Cook & Gao (1996) suggested. In the laboratory experiment, Komai & Emura (1955) showed that the growth rate of the *trans* heterozygote (C^+B^-/C^-B^+) is higher than those of C^+B^-/C^+B^- and C^-B^+/C^-B^+ and that C^+B^-/C^-B^+ and C^-B^+/C^-B^+ may be more resistant to low temperature than C^+B^-/C^+B^-. Thus, this genetic polymorphism for shell patterns may be maintained by overdominance or associative overdominance at loci linked to the C and B loci. It is only case in which the advantage of heterozygote at the ground colour and banding loci was shown by direct comparisons of life history traits between genotypes. In those experiments, Komai & Emura (1955) compared the three genotypes obtained from 75 pairs of DB parents in careful designs of statistics, but did not indicate the origins of those DB parents. For that reason, Clarke (1978) and Clarke et al. (1978) claimed that the superiority of the trans-heterozygotes may have resulted from crosses of DB parents from different populations. However, statistical comparisons were done between the three genotypes within parental crosses. Thus, the confounding effects of crosses between populations cannot explain the heterozygous advantage detected.

If only the *trans* heterozygote (C^+B^-/C^-B^+) is superior, and the other genotypes do not differ in fitness under the linkage disequilibrium, the DU and LB chromosomes would be expected to be frequently common in populations. However, the LU chromosome is overwhelmingly predominant in most populations in Japan, and no cline has been detected (Komai & Emura, 1955; Asami & Ohba, 1982). Thus, the *trans* heterozygote may be superior because of homozygous disadvantages that are specific to the DU and LB chromosomes.

Bradybaena pellucida is also polymorphic in ground colour and banding within populations, where the C^+ allele is dominant to the C^- and the B^+ allele to the B^- as well (Asami et al., 1993, 1997a, b). The close similarities of *B. similaris* and *B. pellucida* as sibling species in morphology and ecology suggest that the four morphs of the latter species are also controlled by a supergene. Comparative studies of the genetic basis of shell polymorphism in these species would be useful to investigate the evolution of the supergene.

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REFERENCES

ASAMI, T., & S. OHBA, 1982. Shell polymorphism in the land snail *Bradybaena similaris* in the Kanto District. — Scientific Report of the Takao Museum of Natural History 11: 13–28.

ASAMI, T., & K. OHBAYASHI, 1999. Effects of oviposition substrate on lifetime fecundity of the terrestrial pulmonate *Bradybaena similaris*. — Journal of Conchology 36: 1–9.

ASAMI, T., H. FUKUDA & K. TOMIYAMA, 1993. The inheritance of shell banding in the land snail *Bradybaena pellucida*. — Venus 52: 155–159.

ASAMI, T., K. OHBAYASHI & K. SEKI, 1997a. The inheritance of shell color in the land snail *Bradybaena pellucida*. — Venus 56: 35–39.

ASAMI, T., H. YAMASHITA, J. PARK & H. ISHIKAWA, 1997b. Geographical distribution of the land snail *Bradybaena pellucida* (Pulmonata: Bradybaenidae). — Yuriyagai 5: 31- 42

ASAMI, T., R.H. COWIE & K. OHBAYASHI, 1998. Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. — American Naturalist 152: 225–236.

CAIN, A.J., 1983. Ecology and ecogenetics of terrestrial molluscan populations. In: W.D. RUSSELL-HUNTER, ed., The Mollusca 6: 597-647. Academic Press, Orlando, Florida.

CAIN, A.J., J.M.B. KING, & P.M. SHEPPARD, 1960. New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L. — Genetics 45: 393–411.

CAIN, A.J., P.M. SHEPPARD, & J.M.B. KING, 1968. The genetics of some morphs and varieties of *Cepaea nemoralis* (L.). — Philosophical Transactions of the Royal Society of London (B) 253: 383–396.

CLARKE, B., 1978. Some contributions of snails to the development of ecological genetics. In: P. F. BRUSSARD, ed., Ecological genetics: The interface: 159-170. Springer-Verlag, New York.

CLARKE, B., W. ARTHUR, D.T. HORSLEY, & D.T. PARKIN, 1978. Genetic variation and natural selection in pulmonate mollusks. In: V. FRETTER & J. PEAKE, eds., Pulmonates 2A: 219-270. Academic Press, London.

COOK, L.M., 1998. A two-stage model for *Cepaea* polymorphism. — Philosophical Transactions of the Royal Society of London (B) 353: 1577–1593.

COOK, L.M., 2005. Disequilibrium in some *Cepaea* populations. — Heredity 94: 497–500.

COOK, L.M., 2007. Heterosis in *Cepaea*. — Biological Journal of the Linnean Society 90: 49–53.

COOK, L.M., & G. GAO, 1996. Test of association of morphological variation with heterozygosity in the snail *Cepaea nemoralis*. — Heredity 76: 118–123.

COOK, L.M., & J.M.B. KING, 1966. Some data on the genetics of shell character polymorphism in the snail *Arianta arbustorum*. — Genetics 53: 415–425.

COOK, L.M., & J. MURRAY, 1966. New information on the inheritance of polymorphic characters in *Cepaea hortensis*. — Journal of Heredity 57: 245–247.

COOK, L. M., R.H. COWIE, & J.S. JONES, 1999. Change in morph frequency in the snail *Cepaea nemoralis* on the Marlborough Downs. — Heredity 82: 336–342.

COWIE, R.H., 1992. Shell pattern polymorphism in a 13-year study of the land snail *Theba pisana* (Müller) (Pulmonata, Helicidae). — Malacologia 34:87–97.

DARBISHIRE, A.D., 1905. Professor Lang's breeding experiments with *Helix hortensis* and *H. nemoralis*; an abstract and review. — Journal of Conchology 11: 193-200 [see LANG, 1904].

DAVISON, A., & B. CLARKE, 2000. History or current selection? A molecular analysis of 'area effects' in the land snail *Cepaea nemoralis*. — Proceedings of the Royal Society of London (B) 267: 1399–1405.

GOODHART, C.B., 1987. Why are some snails visibly polymorphic, and others not? — Biological Journal of the Linnean Society 31: 35–57.

HAYASHI, M., & S. CHIBA, 2004. Enhanced colour polymorphisms in island populations of the land snail *Euhadra peliomphala*. — Biological Journal of the Linnean Society 81: 417–425.

HONEK, A., 2003. Shell-band color polymorphism in *Cepaea vindobonensis* at the northern limit of its range. — Malacologia 45: 133–140.

IKEDA, K., & S. EMURA, 1937. The triple-allelic gene in *Bradybaena similaris stimpsoni*. — Zoological

Magazine 49: 123–124.
JONES, J.S., B.H. LEITH & P. RAWLINGS, 1977. Polymorphism in *Cepaea*: A problem with too many solutions? — Annual Review of Ecology and Systematics 8: 109–143.
KOMAI, T., & S. EMURA, 1955. A study of population genetics of the polymorphic land snail *Bradybaena similaris*. — Evolution 9: 400–418.
LANG, A., 1904. Ueber Versuche zu Untersuchungen über die Varietätenbildungen von *Helix hortensis* Müller und *Helix nemoralis* L. — Denkschriften Medizinisch- Naturwissenschaftliche Gesellschaft zu Jena 11: 439–506 [see DARBISHIRE, 1905].
MURRAY, J., 1963. The inheritance of some characters in *Cepaea hortensis* and *Cepaea nemoralis* (Gastropoda). — Genetics 48: 605–615.
MURRAY, J., 1975. The genetics of Mollusca. In: R.C. KING, ed., Handbook of genetics 3: 3–31. Plenum Press, New York.
MURRAY, J., & B. CLARKE, 1966. The inheritance of polymorphic shell characters in *Partula*. — Genetics 54: 1261–1277.
MURRAY, J., & B. CLARKE, 1976a. Supergenes in polymorphic land snails I. *Partula taeniata*. — Heredity 37: 253–269.
MURRAY, J., & B. CLARKE, 1976b. Supergenes in polymorphic land snails II. *Partula suturalis*. — Heredity 37: 271–282.
OZGO, M., 2005. *Cepaea nemoralis* (L.) in southeastern Poland: association of morph frequencies with habitat. — Journal of Molluscan Studies 71:93–103.
UESHIMA, R., & T. ASAMI, 2003. Single-gene speciation by left-right reversal. — Nature 425: 679.