

## SHELL POLYMORPHISM IN THE LAND-SNAIL *CEPAEA NEMORALIS* (L.) ALONG A WEST-EAST TRANSECT IN CONTINENTAL EUROPE

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**ABSTRACT:** The present study analyses variation in morph frequencies of *C. nemoralis* along a west-east transect spanning the whole range of the species in continental Europe. The transect passed along the 52°N parallel from the North Sea coast in the Netherlands to the Bug River in Poland (1,215 km). Samples were collected at the crossings of the parallel with consecutive meridians, within the radius of 15 km from the crossing point. The material comprises 27,666 individuals from 235 populations. *C. nemoralis* occurred along the whole transect, but was much more common and abundant in its eastern part. In large areas of the western part of the study area it was extremely rare and un abundant, indicating the severity of human impact on the environment, posed especially by industrial agriculture. Variation in morph frequencies did not show clear overall trends; there were some regularities but for the most part they did not coincide with large-scale climatic parameters. There were two significant trends: pink and mid-banded tended to increase eastwards and correlated positively with the measures of continentality. While the trend for mid-banded might be explicable on the grounds of climatic selection, for pink it is directly contrary to expectations and contrasts with the patterns of spatial distribution and temporal changes in other parts of Europe. Probably, the distribution of morph frequencies reflects foremost the founding events occurring during colonisation of novel habitats and range expansion. Contrary to the large-scale variation, the habitat effect consistent with the climatic selection hypothesis was observed along the whole transect, with light shells being more frequent in open than in shaded habitats. As thrush anvils were rare in the study area, and in places where they were present no evidence of selective predation was found, this morph distribution resulted most probably from microclimatic selection. *C. nemoralis* inhabited predominantly anthropogenic, short-lived habitats, and a clear habitat effect observed throughout the study area indicates a high rate of adaptation of populations of this species to environmental conditions. Although expected on theoretical grounds, there was no decrease in the level of polymorphism towards the limits of the species range, and the differences between open and shaded habitats were not consistent in direction. The probable explanation lies with the fact that the current limits of the distribution of this species in continental Europe are not really ecologically marginal. Additionally, the specific mating system protects populations of this species from the impoverishment of the gene pool. Linkage disequilibria were common in the study area. Tight linkage between the loci controlling the colour of the shell and the presence or absence of banding limits the scope of genotypes available to selection, and might be one of the reasons why different populations adapt along different genetic routes to similar environmental conditions. This is probably an additional factor contributing to the maintenance of polymorphism in this strikingly variable species.

**KEY WORDS:** adaptation, climatic selection, evolutionary ecology, founder effects, microclimate, natural selection, range expansion, microevolution



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## 1. INTRODUCTION

The ways in which populations adapt to their environments, and the processes maintaining intraspecific variation are among the central problems in evolutionary biology. Solving them requires connecting genotype with phenotype for traits of possible ecological importance. In most cases, this cannot easily be done, mainly because many fitness-related traits are quantitative and do not have a simple genetic basis. Colour polymorphisms are exceptional in that the genetic component of variation is – at least potentially – clearly discernible. Not surprisingly, animals with varying body coloration are typical model systems, and much of our understanding of the evolutionary processes occurring at population level comes from the study of those organisms (e.g. FORSMAN 1995, GAMBLE et al. 2003, HOEKSTRA et al. 2005, CAESAR et al. 2007, WANG & SHAFFER 2008, NOSIL 2009). However, in most cases the genetic background of variation is assumed rather than certain (e.g. KARK et al. 1997, HARLEY et al. 2006, MCKILLUP & MCKILLUP 2008, UNSICKER et al. 2008, WOOLBRIGHT & STEWART 2008). The systems in which the mode of inheritance of various components of variation is known are few (e.g. BROOKS 2002, ELLERS & BOGGS 2002, MULLEN et al. 2009) but only these allow the insights into the roles of genetic architecture and genetic variation in driving specific elements of adaptation.

Among the most important model organisms of this kind are the land snails of the genus *Cepaea*. Their striking shell variation has long attracted the attention of biologists. Thanks to the work of the early researchers, the mode of inheritance of shell colour and banding in *C. nemoralis* and *C. hortensis* is well understood (reviews in MURRAY 1975, JONES et al. 1977, COOK 1998). This provides the basis for the interpretation of the spatial and temporal patterns of morph composition observed in populations of those species. Additionally, these snails have many advantages as study systems in population biology: wide ecological tolerance resulting in a broad geographical range and the variety of habitats they inhabit, the fact that they often live in distinct, relatively isolated populations (colonies) often reaching high population densities, and low mobility shared with other land snails. Of the two species, *C. nemoralis* is more ubiquitous and more variable, and has been subject to more study. The present study focuses on this species.

*C. nemoralis* is a medium-sized (shell diameter about 20 mm) land snail widely distributed in Western and Central Europe (KERNEY & CAMERON 2006). Its natural distribution range extends from the British Isles in the north to the Iberian Peninsula and Croatia in the south. The eastern limit of its natural range is harder to determine. It is found in natural habitats in north-western Poland (RIEDEL 1988), but it is also

found farther east and south. It presently occurs throughout Poland but almost exclusively in anthropogenic habitats (SULIKOWSKA-DROZD 2008) to most of which it was most probably introduced. Its spread in the Czech Republic is well-documented (HONEK 1995, DVOŘÁK & HONEK 2004), and the easternmost populations of this species are known from Latvia and Estonia in the north (SILVERTOWN et al. 2011) and Lvov in Ukraine in the south (SVERLOVA 2002). These eastern populations are predominantly in urban habitats; the confinement to urban environments is dispersal-rather than climate-limited (OZGO & BOGUICKI 2011).

The species has a wide ecological tolerance. It inhabits a variety of habitats ranging from sand dunes to deciduous forests (JONES et al. 1977), and a broad altitudinal range, reaching as high as 2,100 m a. s. l. in the Pyrenees (ARNOLD 1968). In most of its distribution range it also occurs in anthropogenic habitats: gardens, orchards, cemeteries, railway margins, road verges, derelict sites, unmanaged parts of city parks, or margins of garbage dumps.

*C. nemoralis* is the most polymorphic species among the European land snail fauna (CAIN 1977), with more than 200 recognised shell colour and banding variants (GOODHART 1987). Figure 1 presents the most commonly occurring phenotypes. The ground colour of the shell can be yellow, pink or brown, with various genetically determined shades. There can be up to five usually dark bands; other possibilities include orange or transparent bands. Although any conceivable pattern of banding does in fact occur in nature, the majority of snails are unbanded – 00000, midbanded – 00300, three-banded – 00345, and five-banded – 12345. The bands are numbered 1, 2, 3, 4, 5 from the top of the whorl downwards. A missing band is denoted by 0. Thus, a five-banded shell is scored as 12345, and a shell with only the middle band present, as 00300. Any of the bands can fuse together; in an extreme case almost the whole shell is covered with completely fused bands. Fusion of bands is indicated by brackets. A shell scored as 103(45) is a shell with the second band missing, and bands 4 and 5 fused together; a shell scored as (12345) is a five-banded shell with all bands fused.

This variation has a well known genetic basis (reviews of the inheritance of major morphs in WOLDA 1969, MURRAY 1975, JONES et al. 1977, COOK 1998). The allele for brown is dominant to pink which is dominant to yellow. At another locus which is tightly linked to the colour locus, unbanded is dominant to banded. There are several epistatic interactions among banding genes. Unbanded masks the expression of any banding patterns. If an individual is recessive at this locus, other genes determine the actual



Fig. 1. The major colour and banding morphs of *Cepaea nemoralis*. Photo: M. OŻGO

banding of the shell. At the mid-banded locus which is not linked to colour, mid-banded allele is dominant, and epistatic to more bands. At the three-banded locus, not linked to colour nor mid-banded, the three-banded allele is dominant, and epistatic to more bands. The five-banded condition is shown only in individuals that are recessive at the unbanded, mid-banded and three-banded loci. The rare banding patterns, such as 10345, 12300 or 00045 probably result from variable expression of dominance dependent on modifiers whose genetics is poorly understood (WOLDA 1969). Fusion of bands is multifactorially controlled (CAIN et al. 1960, WOLDA 1969), and the possible effects of ecophenotypic variation have not been examined. The tight linkage between the colour and banding locus, with a recombination rate of less than 1% (LAMOTTE 1954, COOK & KING 1966), results in linkage disequilibria commonly occurring in populations of *C. nemoralis*.

Nearly all populations of *C. nemoralis* studied are polymorphic at more than one locus, but morph frequencies vary greatly among them. This extraordinary variation was initially regarded as non-adaptive and resulting solely from chance events (review in CAMERON 1997). The classical work of CAIN & SHEPPARD (1950, 1954, SHEPPARD 1951) showed that natural selection can affect morph frequencies in populations of these snails. Since then the relative roles of chance events and different selective pressures in shaping the genetic composition of populations have been the subject of considerable debate (reviews in JONES et al. 1977, COOK 1998, OZGO 2005a, 2008, MILLSTEIN 2008, 2009). Presently it is broadly accepted that while founding events and genetic drift play an important role in shaping morph frequencies in populations of *C. nemoralis*, directional selection can be exerted by visually hunting predators, and by regional and local climatic conditions (reviews in JONES et al. 1977, COOK 1998, OZGO 2005a, 2008).

These two agents, visually hunting predators and climate, are predicted to have different effects. Visually hunting predators, predominantly birds, should tend to choose those snails whose shells are most conspicuous against the background; the resulting selection leads to increased frequencies of dark and unbanded shells in dark and shaded habitats with uniform backgrounds, and increased frequencies of light and banded shells in open and variable habitats (CAIN & SHEPPARD 1950, 1954, SHEPPARD 1951, reviews in CAIN 1983, CLARKE et al. 1978, JONES et al. 1977). The hypothesis of climatic selection is based mainly on the fact that coloration in ectotherms has important effect on thermal performance (TRULLAS et al. 2007). Under cool conditions, dark individuals tend to absorb solar radiation more rapidly and reach higher internal temperatures, with a beneficial effect on activity, growth and fecundity. In warm environ-

ments however, they are prone to overheating, and can be at a disadvantage compared to light individuals. The effect of melanisation on thermal performance has been shown in various species of ectotherms (BURLA & GOSTELI 1993, VENCES et al. 2002, ELLERS & BOGGS 2004, TANAKA 2007, LINDSTEDT et al. 2009). In *C. nemoralis*, experimental studies have shown that yellow shells and unbanded shells have greater reflectance in the visible and infrared spectra than pink and banded shells (CHANG 1991), and the internal temperatures of pink or banded snails are significantly higher for a given level of solar radiation (HEATH 1975). Darker snails lose more weight through dehydration when exposed to sunlight (CHANG 1991) and suffer higher differential mortality under severe heat load (RICHARDSON 1974). In both cases, effective selection should result in morph frequencies differing among habitats, with the difference that while shaded environments are predicted to have lower frequencies of yellow shells than open habitats in both, visual selection should result in higher frequencies of banded shells in the open, while the reverse applies in the case of thermal selection. Examples of both can be found (OZGO 2005b, COOK 2008, CAMERON & COOK 2012) but they are not universal and vary in strength among regions.

Thermal selection should also be affected by macroclimate. *C. nemoralis* has a distribution spanning a wide range of climatic regimes, from sub-Mediterranean to near-boreal, and from extreme Atlantic to continental. In the over a century-long history of the research of *C. nemoralis* polymorphism, there have been only two attempts at surveying the composition of populations at the scale of the range of the species. JONES (1973) and JONES et al. (1977), compiling the results of earlier studies, focused only on the frequencies of yellow shells, which increased from the north to the south of Europe, and showed a clear positive correlation with July temperatures. More recently, the Evolution MegaLab (SILVERTOWN et al. 2011) compiled the great majority of past records, and surveyed large parts of the continent with the help of thousands of volunteers collecting data on the shell colour, the presence or absence of bands, mid-banded and many-banded shells. This study confirmed the increase in the frequency of yellow shells southwards, with some local anomalies, and also showed an increase in the frequency of mid-banded shells over the later part of the 20th century.

In Europe, the climate changes not only from the north to the south, but also from the west to the east, becoming increasingly continental, i.e. characterised by increasing annual temperature variation. *C. nemoralis* suffers considerable mortality when temperature drops below  $-7^{\circ}\text{C}$ , and there is some evidence that yellow shells are more resistant to extremes of both hot and cold temperatures (LAMOTTE 1959). Morph frequencies in populations of *C. nemoralis* might be ex-

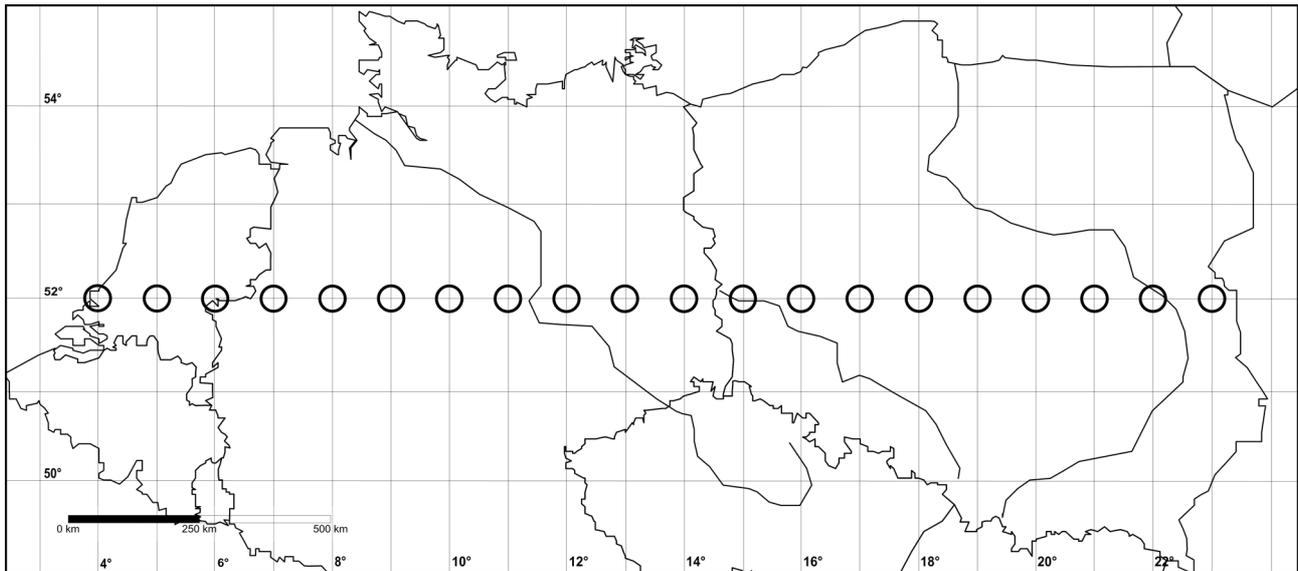


Fig. 2. Map of the study area. Circles indicate the plots in which samples were collected

pected to reflect this transition. The aim of the present study was to analyse in detail the composition of populations of *C. nemoralis* along a transect perpendicular to the transect analysed by JONES (1993) and JONES et al. (1977), spanning the whole natural range of the species from the west to the east of Europe. The transect chosen for this study passes along the 52°N parallel in continental Europe, from the North Sea coast in the Netherlands to the Bug River in Poland (Fig. 2). The 52°N parallel was chosen because it is the longest parallel within the natural distribution range of the species, and it passes mostly through lowland areas, so the effect of altitude on morph frequencies is minimised. The main themes of this study are:

## 2. MATERIAL AND METHODS

The samples were collected along the 52°N parallel in continental Europe, from the North Sea coast in the Netherlands to the Bug River in Poland; the length of this transect was 1,215 km (Fig. 2). The collections were made in the years 2005–2009. Sampling plots were designated at the crossings of the parallel with consecutive 1° longitudinal meridians. Each plot encompassed the area within the radius of 15 km from the crossing point. Throughout the paper the plots are referred to by meridian numbers (degrees east). Prior to the field work, areas potentially suitable for *C. nemoralis* were designated with the help of topographic maps. This excluded highly urbanised areas, coniferous forests, and water bodies. All other environments were searched in the field for suitable habitats, and those were checked for the presence of *Cepaea* snails. Within each study plot, about 10 samples (range 8–15) were collected from areas of about 400 m<sup>2</sup>, covered with relatively uniform vegetation. Habitats were categorised as open or shaded.

the occurrence of *Cepaea* snails along the transect, and – in *C. nemoralis* – the pattern of distribution of morph frequencies along the transect in relation to large-scale climatic parameters and to habitat type, the effect of visual selection on morph frequencies, the amount of among-population variation in relation to position on the transect, variation in the level of polymorphism, distribution of phenotypes encoded by linked and unlinked loci, and the maintenance of polymorphism in relation to linkage disequilibria. The paper is divided into chapters relating to particular aspects of the study with a focused discussion for each. General conclusions are given at the end.

Open habitats were dominated by herbs and grasses, in shaded ones trees and shrubs were dense enough to give shade throughout the day. Latitude and longitude coordinates of each sampling site were taken with a hand-held GPS, and the habitat was photographed. The snails were collected at good daylight conditions; all live snails and well-preserved empty shells were included. At most sites only adult snails were collected. Exceptionally, when snail density was low and it was not possible to collect a sufficient sample of adult snails, large juveniles were also included. After scoring, the snails were returned to the sites of collection. Sample sizes ranged from 23 to 186 (Median 126); together the material comprised 27,666 individuals from 235 populations.

The shells were scored for colour and banding according to the standard system (CAIN & SHEPPARD 1950). The following morphs were included in the analysis: yellow (Y), pink (P), brown (B), unbanded (U), mid-banded (M), three-banded (T), five-banded



(F), and other banding forms (O). Additionally, yellow effectively unbanded (YeU), a genetically amalgamate, but phenotypically relatively uniform group of light-coloured shells was also included. These are yellow shells with two upper bands missing (i.e. unbanded, midbanded, three-banded and some other-banded shells). Brown shells were categorised as unbanded or banded, as banding is only rarely expressed in brown shells. Detailed accounts of the

methods used in analysing different aspects of shell variation are given in the appropriate chapters of this paper. Details of location, habitat and morph composition for all samples are given in Appendix 1. Each sample (site) representing particular population from the plot can be identified in two-digit system – the first denotes the plot number, the second – the sample (population) number within this plot.

### 3. THE AVAILABLE HABITATS AND OCCURRENCE OF *CEPAEA* SNAILS ALONG THE TRANSECT

The transect passes mainly through lowland regions used for agriculture or regions that are partly urbanised. Nearly all habitats were to some degree anthropogenic; not all apparently suitable habitats were occupied by *C. nemoralis*. *C. hortensis* and *C. vindobonensis* also occurred in parts of the transect, and their occurrence might affect that of *C. nemoralis*. The character of each meridian plot and the distribution of *Cepaea* species within it are given below. Plot numbers refer to meridian numbers. Plots 4–6 are in The Netherlands, plots 7–10 in former West Germany, 11–14 in former East Germany, and 15–23 in Poland.

#### Plot 4

The density of human population in the area encompassed by this plot was very high, and the land was highly developed. Areas of intensive agriculture, extensive glass-houses, industrial areas, stud farms, stadiums, roads, high-ways, recreational areas, and human settlements occupied almost the entire area. While the predominant part of the landscape was appropriated by man, the remaining semi-natural habitats were not very highly degraded, and contained a reasonable variety of plant and invertebrate species. *C. nemoralis* was present in only some parts of the plot, and most of the apparently suitable habitats were not inhabited. In areas where the species was present, it occurred in road verges, derelict sites, and less maintained parts of city parks. *C. hortensis* was not found.

#### Plot 5

This region was predominantly agricultural, with a high proportion of pastures. The habitats available to the snails were mostly wooded areas and road-verges. Often vegetation in such places was luxurious, and there was a variety of invertebrates. *C. nemoralis* occurred commonly, and occupied many suitable habitats. *C. hortensis* was not found.

#### Plot 6

This plot encompassed pine forests, agricultural areas and densely built-up human settlements. Agriculture was for the most part very intensive, with the

predominance of plough-land. Only in the north-eastern part of the plot the landscape was mosaic-like, and pastures common; this area was also in tourist and recreational use. The habitats available to the snails were mostly road-verges and wooded areas, which usually contained a variety of plant and invertebrate species. In this part of the plot *C. nemoralis* was rather common. It occurred predominantly in road verges bordering on pastures. Road verges along arable fields, including grass plantations, were inhabited less frequently and the snails were less abundant. In areas under intensive agricultural use *C. nemoralis* occurred only sporadically and at low population densities. *C. hortensis* was not found.

#### Plot 7

This study plot was located in a very intensively managed region. The landscape consisted of industrial agriculture, roads, and densely built-up human settlements. Vegetation in road verges was usually cut very short. In those ditches in which it was allowed to grow, it was dominated by just one species, most often *Urtica dioica* or *Origanum vulgare*. Cities and towns were cleared of any unmanaged places; there were no *Cepaea* in the parts of city parks which were not manicured, and therefore provided what appeared like suitable habitats. In the whole area, absence of invertebrates from the remnant patches of spontaneous vegetation was striking. *C. nemoralis* was absent from almost the whole study plot. In spite of a thorough search, the species was found in only one small area in the north-east of the plot. Here, the countryside was somewhat less managed, forming a mosaic of arable fields, small patches of woods, orchards, and hedges; the fields were separated by baulks grown for the most part with nettles, but also some herbs and scrub. *C. nemoralis* occurred predominantly in the nettles, sometimes in high numbers. It less often inhabited the hedges, and was not numerous there. It was absent from apparently suitable shaded habitats, such as woods or orchards, even when they were directly adjacent to the open sites inhabited by the snails. There were both *C. nemoralis* and *C. hortensis* in this part of the plot, but they occurred separately.

#### Plots 8, 9 and 10

These plots were located in a highly managed region where in the lowlands intensive agriculture predominated. In such countryside, road verges and ditches can provide suitable habitats for *C. nemoralis*. In this region, however, they were usually cut very short. Short grass is not a suitable habitat for these snails; more importantly, cutting itself is a cause of very high mortality. In places where vegetation was more luxuriant, it was usually dominated by one species, most often *Urtica dioica* or *Oreganum vulgare*. Absence of invertebrates was striking. On the whole, however, the countryside was less managed and more mosaic-like than in plot 7, and patches of spontaneous vegetation provided potential snail habitats. *C. nemoralis* was absent or very rare in many parts of these plots, and the majority of apparently suitable habitats were not inhabited. Where present, the snails most often occurred in very localised populations, occupying only small parts of available habitats, for example they were present in a single bush, but not in other similar bushes in the vicinity, or only in a small part of an extensive area grown with nettles. Typically, the snails would be present in a road verge on one side of the road, but not on the other, even though it looked very much the same, or they would be present in the road verge, but not in the hedge directly adjacent to it. The numbers of snails in such populations were often very low. *C. hortensis* was not found in plot 8. In plot 9 it was present and abundant at two sites, at which there were no *C. nemoralis*. In plot 10 it was found at 9 sites, in 7 of which it occurred together with *C. nemoralis*.

#### Plots 11, 12, 13 and 14

These plots were located in a region with clearly less intensive human impact than those to the west, which coincided with the border between the former west and east Germany. Favourable *Cepaea* habitats, such as unmanaged parks and cemeteries, orchards, derelict sites, railway margins and road verges with luxuriant vegetation were common. In many parts of these plots *C. nemoralis* was relatively easy to find and abundant. In most cases suitable habitats were colonised. In plot 11, *C. hortensis* was also present and locally abundant. It often co-occurred with *C. nemoralis*. In plots 12, 13 and 14 *C. hortensis* was also present but much more rare. When present, it usually co-occurred with *C. nemoralis*, but at very low densities.

#### Plot 15

This study plot was located in an area with very little human activity. The landscape was mosaic-like, with fields, meadows and pastures interspaced with baulks, hedges, woods, abandoned human settlements, old orchards, cemeteries etc. This provided an abundance of favourable *Cepaea* habitats. The plot was clearly divided into areas occupied by *C. nemoralis*,

where *C. hortensis*, when present, was very rare, and areas occupied by *C. hortensis*, from which *C. nemoralis* was practically absent. In the respective areas of occurrence each of the species was abundant and occupied most of the suitable habitats. Strikingly, no differences in the habitat between areas occupied by those species were apparent.

#### Plots 16, 17, 18 and 19

The region in which these study plots were located was predominantly in agricultural use. Although human activity was quite high, there was still an abundance of wildish places available to the snails, both in rural and in urban environments. In plots 16 and 17, both species of *Cepaea* were common and abundant. However, the areas with the predominance of either of them only partly overlapped, and as in plot 15, no differences between areas occupied by *C. nemoralis* and *C. hortensis* were apparent. In plot 17, it was *C. hortensis* that was more wide-spread and abundant. It was common also in towns where it occupied habitats typical of *C. nemoralis* in other areas: derelict sites, road verges, or cemeteries. *C. nemoralis* was rarer and occurred in localised populations. In plot 18, *C. nemoralis* was wide-spread and abundant. Most of suitable habitats were colonised, both in urbanised and in rural areas. *C. hortensis* was present at only one site, in co-occurrence with *C. nemoralis*. In plot 19 *C. nemoralis* was present mostly in towns, where it occupied parks, cemeteries, derelict sites, and railway stations. Many suitable habitats were uncolonised, but in places where the snails were present they were abundant. *C. hortensis* was found at only two sites, in co-occurrence with *C. nemoralis*.

#### Plots 20, 21 and 22

Human impact on natural environment traditionally has been lower in eastern than in western Poland. Presently, the region in which these plots are located is in agricultural, horticultural and fruit-farming use, but unmanaged areas are abundant. Also, with the exception of highly urbanised areas, there are many unmanaged places in cities and towns. In these plots *C. nemoralis* was wide-spread and abundant, present in urban, rural and natural environments. Populations usually occupied whole suitable habitats, which were often extensive. Very striking was the almost ubiquitous occurrence of the species in plots 21 and 22 (with the exception of areas with poor sandy soil that lacked suitable habitats). In plot 20, *C. hortensis* was found at just one site, in which there was no *C. nemoralis*. In plot 21, no *C. hortensis* were found, but *C. vindobonensis* was locally present in co-occurrence with *C. nemoralis*. In plot 22, only *C. nemoralis* was found.

#### Plot 23

Land use patterns and landscape structure in this plot did not differ in any apparent ways from plots 20,



Fig. 3. Examples of typical habitats of *Cepaea nemoralis*: A, B, C – open; D, E, F – shaded; samples nos 7.7, 4.3, 19.12, 13.9, 15.12, 18.11, respectively. Photos: M. OZGO

21, and 22. There was however a big difference in the occurrence of *Cepaea* snails. *C. nemoralis* occurred only in urban environments, was rare and formed very localised populations. *C. vindobonensis* was wide-spread and abundant. It was present in natural and semi-natural habitats, and also in such urban environments that are typically occupied by *C. nemoralis* in other regions. *C. hortensis* was also present and lo-

cally abundant. At some sites, all three species occurred together.

#### DISCUSSION

This survey showed that *C. nemoralis* occurred, with varying abundance, along the whole transect. The distribution of *C. hortensis* differed from that of *C. nemo-*

*ralis* in that it was more insular, with centres of abundance in plots 10 and 15 to 17. Although the issue of competition among land snails is a contentious topic (CAIN 1983, TILLING 1985a, b), there is evidence that complete districts within the range of both *C. nemoralis* and *C. hortensis* may be dominated by one or the other species (CAIN 1983). In this study, *Cepaea* species co-occurred at many sites, but the presence of the so-called *nemoralis*-countries and *hortensis*-countries (sensu CAIN 1983) was evident. As no environmental differences between those countries were apparent, these findings support the hypothesis of mutual exclusion of those species. The possible mechanisms of such exclusion remain a mystery (CAIN 1983). *Cepaea vindobonensis* was wide-spread and abundant only in the easternmost plot; apart from this it was locally present in plot 21. As with *C. hortensis*, it appeared to occupy habitats also suitable for *C. nemoralis*, suggesting that the first species to reach a habitat may exclude its congeners (R. A. D. CAMERON, pers. comm.).

The distribution of *C. nemoralis* along the transect was somewhat different from expectations: in western parts of the transect, i.e. in areas within the natural range of the species, it was often hard to find, whereas in the more easterly parts it was common and abundant in many places. The reasons for this pattern of distribution are probably related on the one hand to the patterns of land use, and on the other to the ease with which these snails are transported by humans.

At the western end of the transect, plot 4 was located in an area in which approximately 50 years ago large-scale drainage of marshlands and land development had begun. Marshlands are unsuitable for *C. nemoralis*, so most probably it was only recently introduced, and its localised occurrence indicates that it is currently colonising areas which have become suitable. At the eastern end, in plot 23 it occurred only locally and only in urban environments, which most probably indicates that it was introduced recently and is spreading in available habitats. Within the extremes

of the transect, *C. nemoralis* is considered to be widely distributed (KERNEY & CAMERON 2006). It is a typical eurytopic species, able to tolerate a wide range of ecological conditions, and colonise a variety of habitats. It is also synanthropic, able to persist at sites which might be described simply as wildish places within anthropogenic environment. Figure 3 presents typical *Cepaea* habitats in contemporary continental Europe. The species readily colonises available habitats and recovers from drastic reductions in population numbers (MURRAY 1964, OŹGO & BOGUICKI 2011). In sum, it is a common and undemanding species.

This survey shows, however, that even such a species can be eradicated by human activity. In the most highly managed areas, the scarcity of suitable habitats, and the absence of the species from the habitats that remained, was striking. It can hardly be explained by some previously unreported disjunction in the natural occurrence of the species. The fact that its absence or rarity was observed in the most heavily impacted areas cannot be regarded as coincidental. The most probable reasons for the eradication of the species from areas dominated by industrial agriculture are, on the one hand, appropriation of almost whole landscape for food and bio-fuel production, and on the other hand, the often heavy use of pesticides and nutrients. The striking absence of other invertebrates from habitats potentially suitable for *C. nemoralis* confirms this view.

The current biodiversity crisis concerns not only species that are rare and vulnerable; increasingly we are losing species that are common and wide-spread (GASTON & FULLER 2007, 2008). *C. nemoralis* can be regarded as an indicator of the health of the part of nature usually regarded as common, not especially valuable and generally not endangered. The absence of this species from large areas within its natural range is indicative of an exceptionally severe damage to the environment.

#### 4. MORPH FREQUENCY VARIATION ALONG THE TRANSECT AND ASSOCIATION WITH CLIMATIC VARIABLES

Geographic variation in heritable traits may provide evidence for natural selection, especially if it can be linked to parallel variation in environmental factors. If natural selection occurs, geographic variation in the selective factor will give rise to parallel geographic variation in the traits selected for. Conversely, unselected traits will vary independently of environmental variables (ENDLER 1986). The latitudinal cline in frequencies of yellow shells related to July temperatures is an excellent example (JONES 1973, JONES et al. 1977, SILVERTOWN et al. 2011).

Although from the population-genetics point of view, *C. nemoralis* is probably the best studied non-human animal species, most surveys of the genetic composition of populations were local in character, and centered on the British Isles; a more general view has been provided by the MegaLab citizen-science project (SILVERTOWN et al. 2011). The sampling design of the present study provides data collected at regular intervals along a west-east line, and enables longitudinal trends to be detected.



## METHODS

The methods of sampling and scoring the snails are described in Chapter 2. In order to avoid the bias resulting from different sample sizes, morph frequencies were averaged over populations within each study plot, and these plot means were used in the analyses. Frequencies often varied greatly among samples in each plot, and the number of samples involved was small. Hence, no attempt was made to test the significance of differences in means between plots; the analysis concentrates on consistent trends among the whole array.

Climatic data were obtained using the New\_LocClim\_1.10 programme made available by the Food and Agriculture Organization of the United Nations at [www.fao.org/nr/climpag/locclim/locclim\\_en.asp](http://www.fao.org/nr/climpag/locclim/locclim_en.asp). This programme provides an estimate of climatic conditions at locations for which no observations are available. To achieve this, the programme uses the global agroclimatic database maintained by the Agrometeorology Group of FAO. With the use of this programme the estimates of climatic conditions were obtained at the intersections of the 52 parallel with consecutive meridians with the method of the nearest neighbour interpolation, and the maximum number of stations set at 10. The mean distance of stations from which data were interpolated ranged from 57 km (maximum 87 km) at the 10th meridian to 154 km (maximum 217 km) at the 19th meridian.

The following climatic variables and indices were included in the analysis: mean annual temperature, mean summer temperature, summer sum of sunshine hours, annual and summer sum of precipitation, mean annual and mean summer potential evapotranspiration (PET), Gorchynski Index of Continentality, De Martonne Index and Radiative Index of Dryness (RID). In this study, summer is understood as the May-August period, because this is the time when snails are active in the whole study area. The Gorchynski Index of Continentality, further referred to as Continentality Index, is the measure of continentality by the amplitude of the annual temperature cycle and the sine of latitude; the De Martonne Index is the annual precipitation sum divided by the annual mean temperature plus 10; the Radiative Index of Dryness is the ratio of local radiation balance and the energy needed to evaporate all precipitation ([www.fao.org/nr/climpag/locclim/locclim\\_en.asp](http://www.fao.org/nr/climpag/locclim/locclim_en.asp)). The estimated climatic parameters for each plot are shown in Appendix 2.

## RESULTS

The composition of the samples and information on the collection sites are given in Appendix 1. Table 1 shows the mean frequencies of the major morphs in each plot by habitat. Frequencies of the colour and banding morphs changed along the transect with cer-

Table 1. The mean frequencies (%) of each morph in each plot, separated by habitat. For brown, the numbers are of samples in which the morph was present

Country	Plot	Total samples	No samples		Yellow		Unbanded		Mid-banded		Three-banded		No with brown	
			open	shaded	open	shaded	open	shaded	open	shaded	open	shaded	open	shaded
NL	4	11	5	6	50.4	43.7	39.4	34.8	21.6	38.0	7.6	13.0	0	2
NL	5	15	8	7	66.7	54.3	29.9	35.9	35.2	40.3	49.6	31.4	8	4
NL	6	11	7	4	65.8	59.0	56.8	66.8	0.8	6.0	63.3	36.8	3	3
DE	7	8	5	3	81.3	84.7	15.5	0.8	15.5	20.5	18.2	4.8	0	0
DE	8	10	7	3	65.1	41.3	55.5	43.7	16.4	41.1	25.1	9.5	2	1
DE	9	9	4	5	67.3	76.1	28.2	24.5	14.4	3.0	33.0	13.1	0	0
DE	10	11	5	6	76.8	61.7	24.2	14.5	20.6	3.4	16.2	21.5	0	0
DE	11	13	7	6	73.7	64.1	55.7	34.8	2.6	1.5	63.3	56.4	0	0
DE	12	10	5	5	47.0	37.9	26.0	31.0	32.8	54.2	27.1	18.2	0	0
DE	13	10	4	6	25.7	25.6	46.5	40.7	60.8	45.7	13.6	32.4	2	2
DE	14	10	5	5	37.2	27.4	15.8	30.1	56.9	48.6	15.7	30.7	1	0
PL	15	13	5	8	60.2	26.9	11.8	52.9	39.2	47.4	0.0	0.0	0	1
PL	16	14	7	7	41.2	34.3	19.8	26.6	62.5	54.3	34.2	41.8	0	3
PL	17	13	7	6	70.9	41.5	9.7	26.2	31.7	47.8	5.5	2.5	2	3
PL	18	15	8	7	68.5	59.5	36.5	27.0	48.7	44.2	1.5	8.2	0	0
PL	19	14	8	6	53.5	42.5	20.1	35.9	38.4	31.4	24.5	15.4	3	4
PL	20	14	5	9	41.1	26.0	33.4	31.4	39.6	37.9	45.1	43.5	3	6
PL	21	10	5	5	52.7	39.8	6.9	18.5	65.2	75.2	17.5	10.4	0	1
PL	22	15	7	8	38.8	25.0	18.9	23.5	53.7	45.9	30.0	18.1	0	2
PL	23	9	3	6	23.2	8.0	65.1	39.1	36.8	37.1	37.8	32.1	0	0

tain regularities, showing minima and maxima, but without clear overall trends (Fig. 4). ANOVA (on arcsine transformed frequencies) confirms that for all morphs differences among plots were highly significant despite considerable variation among samples within each (Table 2). The amount of variation within and among plots is analysed in more detail in Chapter 6. Brown shells were rare, and their distribution changed erratically along the transect. Generally, colour and banding changed independently. The only exceptions were pink which was associated with mid-banded (tau=0.505, p=0.002), (Fig. 5A) and yellow, associated with five-banded (tau=0.410, p=0.011), (Fig. 5B). There was no correlation between unbanded and yellow or pink, or between yellow and pink unbanded. In fact, yellow unbanded and pink unbanded as a proportion of the total showed very different patterns of distribution (Fig. 4C).

Correlations of morph frequencies with climatic parameters are given in Table 3. Variation of morph frequencies and climatic parameters is presented in Appendix 3. Pink and mid-banded correlated positively with mean summer temperatures and sunshine hours, and consequently, with potential evapotranspiration (PET), Continentality Index, and Radiative Index of Dryness (RID). Yellow, which were almost complementary to pink, correlated negatively with the same parameters. Yellow unbanded correlated negatively with the sum of sunshine hours and Radiative Index of Dryness, and positively with the sum of summer precipitation, while pink unbanded correlated positively with mean annual temperature. The only correlation shown by yellow effectively unbanded was a negative correlation with the sum of sunshine hours. Brown did not correlate with any of

the climatic parameters, and no morph was correlated with water vapour pressure. Pink and mid-banded, and yellow and five-banded showed associations with the same climatic parameters; this reflects the close co-variation of the frequencies of these morphs along the transect (Fig. 5). It should be noted that the correlations, even when significant, were weak or very weak, with the values of tau coefficient in significant correlations ranging from 0.319 to 0.670.

## DISCUSSION

Populations of *C. nemoralis* sampled along the 52°N showed an immense range of variation. Mean frequencies of all morphs varied significantly with location but did not, in general, show a linear response to longitude. While there were some regularities in this variation, much of it was not associated with large scale climatic variables. There were two significant and similar trends: pink shells and mid-banded within banded shells tended to increase eastwards, and correlated positively with the measures of continentality.

Under the assumptions of the climatic selection hypothesis, the frequencies of yellow shells are expected to correlate positively with summer temperatures and related climatic variables; the trend in pink and its complementary reversal in yellow are directly contrary to expectations. They also contrast with the patterns of spatial distribution and temporal changes in the frequencies of colour morphs in other parts of Europe, particularly with the cline of decreasing frequencies of pink shells from the north to the south of western Europe in association with increasing summer temperatures (JONES 1973, JONES et al. 1977, SILVERTOWN et al. 2011), and with an increase in the

Table 2. Analysis of variance on arcsine transformed frequencies. Residual variance is that occurring within plots. Two samples had no many-banded shells, hence the difference in total for three-banded in banded

Yellow	ss	df	ms	F		% explained
plots	31203	19	1642,263	7,472416	P<0.001	39,8
residual	47252	215	219,7767			
total	78455	234				
Unbanded	ss	df	ms	F		
plots	20243	19	1065,421	4,492714	P<0.001	28,4
residual	50986	215	237,1442			
total	71229	234				
Mid-banded	ss	df	ms	F		
plots	46637	19	2454,579	8,301367	P<0.001	42,3
residual	63572	215	295,6837			
total	110209	234				
Three-banded	ss	df	ms	F		
plots	42817	19	2253,526	7,54873	P<0.001	40,2
residual	63587	213	298,5305			
total	106404	232				

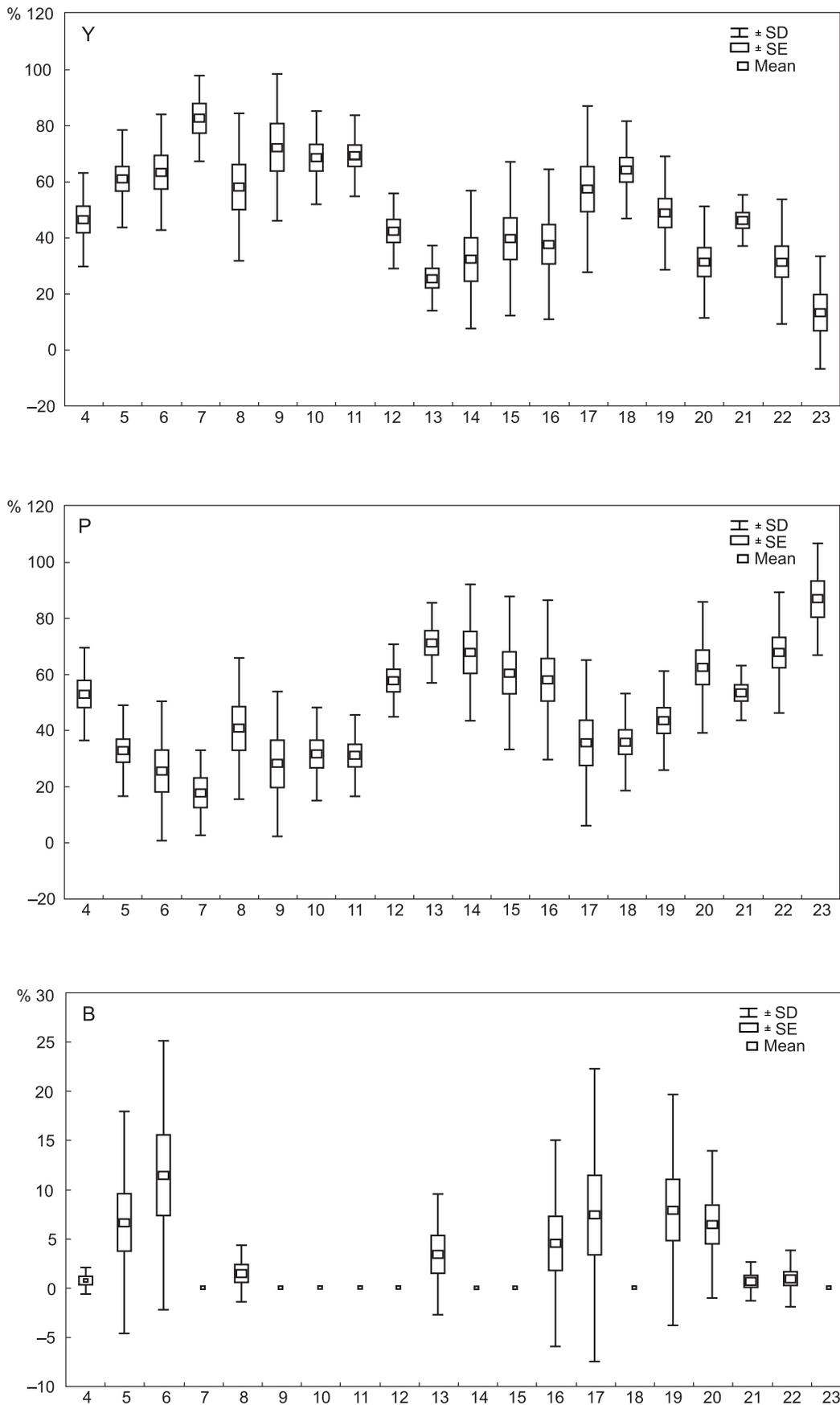


Fig. 4A. Frequencies of the colour and banding morphs along the transect: Y – yellow, P – pink, B – brown; 4–23 – plot numbers

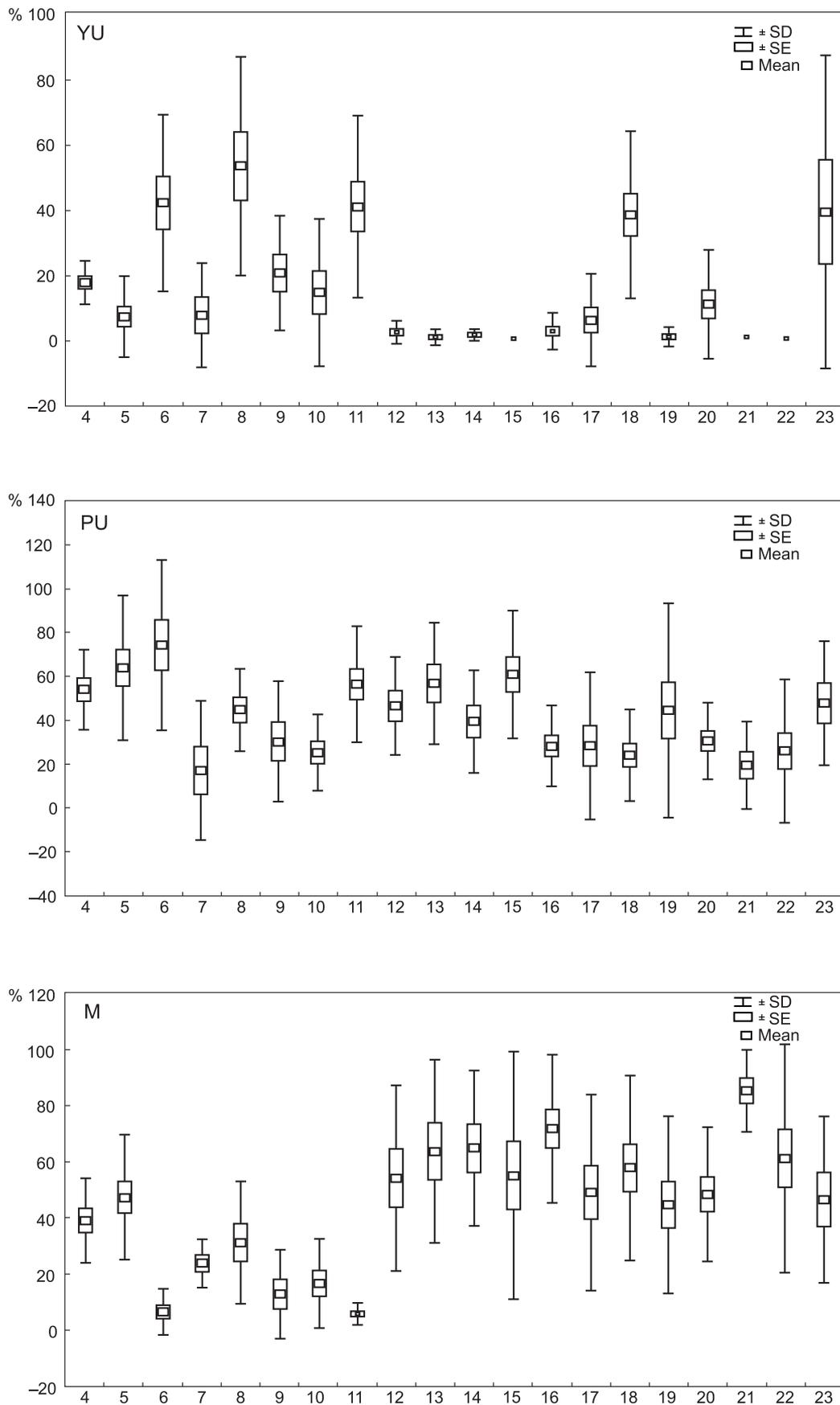


Fig. 4B. Frequencies of the colour and banding morphs along the transect: YU – yellow unbanded, PU – pink unbanded, M – mid-banded; 4–23 – plot numbers

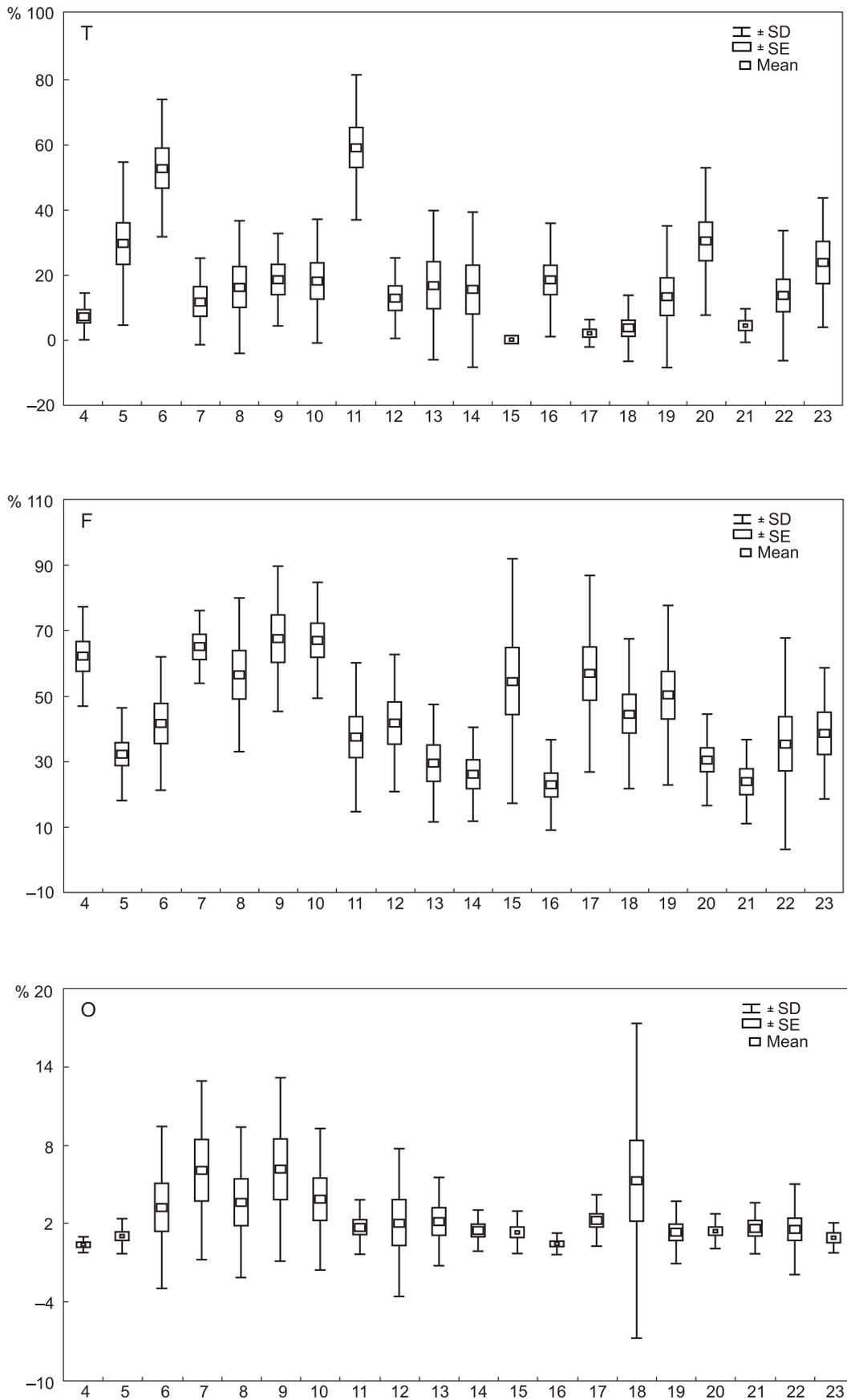


Fig. 4C. Frequencies of the colour and banding morphs along the transect: T – three-banded, F – five-banded, O – other; 4–23 – plot numbers

Table 3. Coefficients of Kendall rank correlation of morph frequencies with climatic parameters

Climatic parameter	Yellow	Pink	Brown	Yellow unbanded	Pink unbanded	Mid-banded	Three-banded	Five-banded	Yellow effectively unbanded
Mean annual temperature	0.239 #	-0.281 #	0.079 #	0.207 #	0.345 *	-0.175 #	0.048 #	0.101 #	0.186 #
Mean temperature May–August	-0.546 ***	0.503 **	0.232 #	-0.171 #	-0.064 #	0.374 *	-0.107 #	-0.353 *	-0.235 #
Sum of sunshine hours May–August	-0.616 ***	0.670 ***	-0.029 #	-0.443 **	-0.022 #	0.497 **	-0.184 #	-0.368 *	-0.465 **
Sum of annual precipitation	0.249 #	-0.260 #	-0.056 #	0.271 #	0.164 #	-0.334 *	0.239 #	0.260 #	0.101 #
Sum of precipitation May–August	0.219 #	-0.229 #	-0.034 #	0.379 *	-0.059 #	-0.432 **	0.229 #	0.325 *	0.165 #
Mean potential evapotranspiration May–August	-0.383 *	0.415 **	0.006 #	-0.167 #	-0.286 #	0.426 **	-0.199 #	-0.189 #	-0.264 #
Water vapour pressure May–August	-0.039 #	0.028 #	0.076 #	0.028 #	-0.215 #	0.039 #	-0.204 #	0.072 #	-0.072 #
Continental Index	-0.426 **	0.468 **	0.085 #	-0.277 #	-0.213 #	0.319 *	-0.085 #	-0.266 #	-0.277 #
De Maratone Index	0.228 #	-0.228 #	-0.063 #	0.239 #	0.076 #	-0.402 *	0.283 #	0.326 *	0.098 #
Radiative Index of Dryness	-0.375 *	0.396 **	0.068 #	-0.332 *	-0.153 #	0.406 *	-0.280 #	-0.290 #	-0.195 #

Statistical significance:

# P&gt;0.05

\* P&lt;0.05

\*\* P&lt;0.01

\*\*\* P&lt;0.001



frequencies of yellow effectively unbanded snails at the expense of brown observed over 43 years in The Netherlands, presumably in adaptation to climatic warming (OZGO & SCHILTHUIZEN 2011). No biologically meaningful explanations for the present results are apparent. A complicating factor is that there is a contradiction within the results: in response to local

climates morph frequencies show clear habitat-effect, with yellow and yellow effectively unbanded more frequent in open than in shaded habitats (see Chapter 5). As open habitats are generally hotter, dryer and have larger temperature variations than shaded ones, higher frequencies of light shelled snails in those habitats are consistent with the hypothesis of climatic se-

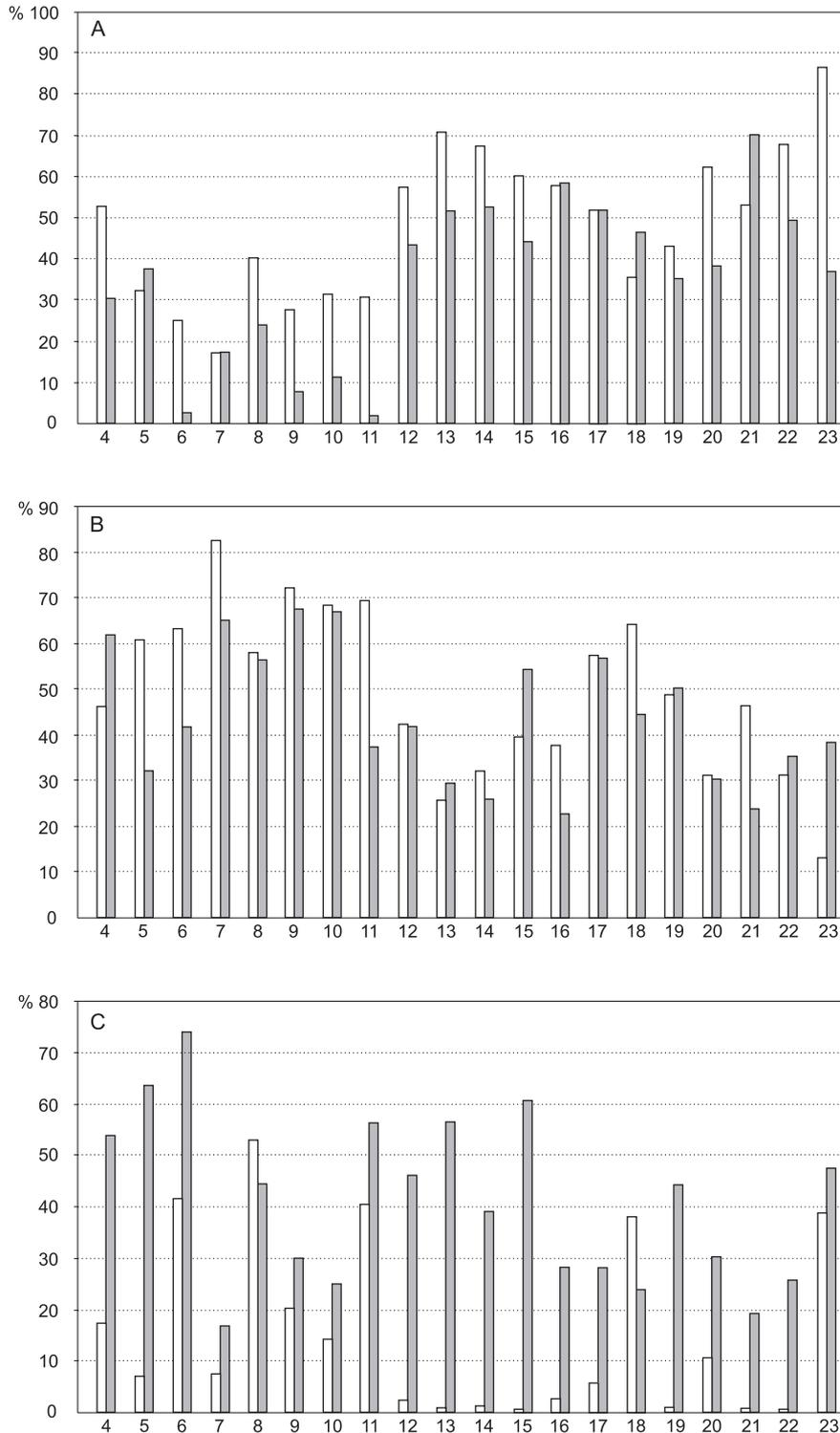


Fig. 5. Distribution of morph frequencies along the transect: A – pink (white bars) and mid-banded (grey bars); B – yellow (white bars) and five-banded (grey bars); C – unbanded in yellow (white bars) and in pink (grey bars). 4–23 – plot numbers

lection. The fact that similar climatic gradients on a larger geographical scale produce opposite effects is difficult to explain.

The frequencies of mid-banded shells showed very similar associations with climatic parameters as pink shells, and this trend is in greater conformity with other results. Mid-banded snails are probably favoured under conditions of great temperature variation (BANTOCK & PRICE 1975), and prolonged drought (WOLDA 1965). These are phenomena characteristic of the continental climate, and the positive associations of mid-banded with summer temperatures, sunshine hours, potential evapotranspiration, Continentality Index, and Radiative Index of Dryness may represent an adaptive response to the increased continentality of climatic conditions. Interestingly, a recent continental-scale analysis of temporal changes in morph frequencies of *C. nemoralis* has shown a significant increase in the frequencies of mid-banded shells in Europe, probably related to the temperature increase (SILVERTOWN et al. 2011). An increase in the frequencies of this morph has also been noted in more local studies of temporal changes in the genetic composition of *Cepaea nemoralis* populations (CLARKE & MURRAY 1962a, b, COOK & PETTIT 1998, CAMERON 2001). In south-eastern Poland, the area characterised by particularly high temperature amplitudes, 94% of shells were banded, and 73% of banded shells were mid-banded (OŻGO 2005a). The reasons for the association of mid-banded shells with temperature and related climatic parameters are not understood, but cannot relate simply to the thermal properties of the shells: yellow unbanded and three-banded, with presumably very similar thermal properties, do not show such associations. Most probably some as yet undiscovered pleiotropic effects of the mid-banded gene are involved.

As the distribution of mid-banded along the transect is consistent with the hypothesis of climatic selection, a conceivable interpretation of the present results might be that the large-geographical-scale climatic selection affects the banding rather than the colour, and the colour, for some reason, co-occurs with the selected for banding morphs. However, there is no known genetic explanation for the co-variation of pink and mid-banded (MURRAY 1975), and there was no within-population trend for mid-banded shells to be commoner in pink than in yellow, and so this interpretation is rather unlikely. There are other reasons for caution in interpreting the associations of morph frequencies with macroclimatic variables. In plot 10, climate was markedly different than in those adjacent to it, presumably due to a higher altitude, yet frequencies were not markedly different among

them. Similarly, within central and eastern Poland frequencies oscillated (Fig. 4) while climatic parameters were either level or changing monotonically.

An alternative or additional explanation of the observed patterns is that the distribution of morph frequencies along the transect results mostly from chance events related to recent colonisation. At least part of the long-distance snail migration occurred with the help of the railway transport, as has been observed in other studies (e.g. HONEK 1995). At the four most easterly study plots, the transect coincided with the major west-east railway line. In this part of the transect, a sharp increase in the frequencies of pink shells occurred. Illustrative is the case of the most easterly town, Biała Podlaska. There, *C. nemoralis* occurred only in the close vicinity of the railway, in small isolated populations. Strikingly, all snails in these populations were pink. In Międzyrzec, located 20 km west of Biała Podlaska and connected with it by the railway, the average frequency of pink was 80%; these were the only two places in plot 23 where *C. nemoralis* was present. In passive transport, the most frequent morphs are most likely to be carried along, and to found subsequent populations; the easterly increase in the frequencies of pink shells can simply be a product of the colonisation process. A similar loss of genetic variation most probably due to stochastic processes during range expansion has been observed in a colour polymorphic damselfly *Ischnura elegans* (GOSDEN et al. 2011). Additionally, when colonising snails originate from various populations, an initial excess of homozygotes will result in an increase in the frequencies of dominant morphs, and thus high frequencies of pink shells may also result from the segregation of alleles in recent colonisers.

The colonisation of the most easterly parts of the range has almost certainly been very recent (RIEDEL 1988). That area had not been surveyed for *Cepaea* before, so an earlier absence of the species cannot be ascertained. However, in places where the snails were strikingly abundant, local people commented that the snails had been present for no more than 10 years. Obviously, at lower densities they may not have been noticed, but the now abundant presence may indeed be very recent. Following introduction, population growth can be explosive and lead to very high local densities, with little change in the overall morph composition (OŻGO & BOGUCKI 2011). If this occurred in the eastern parts of the study area, the current morph frequencies would reflect foremost the founding events, with only minor if any effect of large-scale climatic selection. This explanation, being simpler, may be more realistic than explanations in terms of natural selection for which no biological sense is evident.

## 5. THE HABITAT EFFECT: VISUAL AND CLIMATIC SELECTION

One of the recognised patterns in the distribution of morph frequencies in populations of *Cepaea* snails is the so-called habitat effect. This term refers to a situation when in a given area the frequencies of light shells are higher in the open than in the shaded habitats. Such morph distribution probably results from the operation of natural selection: visual, climatic or a combination of both. Under climatic selection hypothesis, morphs are selected for because of their thermal properties. Dark shells: pink, brown and five-banded, are expected to occur at higher frequencies in shaded than in open habitats, while open habitats are expected to contain higher frequencies of yellow unbanded or only lightly banded shells. Under visual selection hypothesis, the visual properties of the shells are important. Dark unbanded shells, i.e. brown and pink unbanded, are expected to occur at higher frequencies in habitats with dark and relatively uniform backgrounds such as beech woods; yellow and banded shells are expected to occur at higher frequencies in open habitats with varied backgrounds. Banding can provide protection from the attack of a predator in a habitat in which grasses and undergrowth cast a multitude of narrow shades. However, when only the shell colour is considered, both selective pressures are expected to give similar results: higher frequencies of yellow shells in open than in shaded habitats.

The classical studies showing that birds are selective towards the colour and banding of the shells and tend to choose those that stand out most against the background were the first demonstration of the power of natural selection affecting morph frequencies in populations of *Cepaea* snails. These findings have dominated the field and are featured in numerous textbooks (e.g. KRZANOWSKA et al. 1982, 2002, SCHILTHUIZEN 2001, FUTUYMA 2005, BELL 2007, BROMHAM 2008). However, doubts as to the universality of this kind of selection were expressed already early on (e.g. GOODHART 1958, LAMOTTE 1959, WOLDA 1963), and although the effect of bird predation on the composition of *Cepaea* populations has been extensively studied (reviews in e.g. JONES et al. 1977, CLARKE et al. 1978, CAIN 1983), the evidence that bird predation leads to habitat matching is not as strong as often believed (COOK 2008). To date, the relative importance of visual and climatic selection in shaping the habitat effect has not been resolved. In this chapter the habitat effect in the study area is analysed in view of the visual and climatic selection hypotheses.

### METHODS

The general sampling and scoring procedures are described in Chapter 2. Yellow and yellow effectively

unbanded were considered as light shell categories; significance of differences in their frequencies at open and shaded sites along the transect was determined with the Wilcoxon paired-sample test.

Each site where the samples were collected was also searched for shells of *Cepaea* snails broken in a way characteristic of thrush predation. Those are shells with a complete or almost complete lip and a missing part behind it, grouped around a hard object such as a stone, brick, bottle, railway track etc., which the bird used to break open the shells. At most sites at which bird anvils were present all such shells were collected; at sites 13.4, 21.6 and 22.6 predation was massive (Fig. 6) and only a part of the available shells was taken. In order to avoid counting parts of the same shell more than once, only shells with a whole lip were included. At the sites where shells were collected from bird anvils, disintegration of dead shells must have been proceeding fast, as there were practically no other dead shells than the predated ones, and they all looked fresh. Dead shells are a valuable source of calcium for living snails and, especially in acidic environments, are quickly eaten up (OŽGO & BOGUČKI 2006). Thus, it can be safely assumed that the shells present at the anvils were predated by the birds during the same season as the live collection was made.

The predated shells were scored in the same way as live snails. Morph frequencies in the predated shells were compared with shells of live snails collected at the same sites, and the significance of differences was determined with the chi-square test. To test the consistency of differences over all sites, the Wilcoxon paired-sample test was used.

### RESULTS

Habitat had a clear influence on morph frequencies. Along the whole transect, the frequencies of yellow and yellow effectively unbanded were higher in open than in shaded habitats (Figs 7, 8). The differences were significant in both those shell categories (yellow, Wilcoxon paired-sample test  $p=0.000517$ ; yellow effectively unbanded, Wilcoxon paired-sample test  $p = 0.000219$ ), but were more pronounced in yellow effectively unbanded. Brown shells were absent in many samples and plots; within The Netherlands and Germany the morph did not occur more frequently in one habitat than in the other, but in Poland it occurred more frequently in shaded sites ( $\chi^2=5.04$ ,  $P<0.05$ ). There was a tendency for the mid-banded and three-banded to be more frequent in open than in shaded habitats (Figs 9, 10) and for five-banded to be more frequent in shaded than in open habitats (Fig. 11), but the differences were not statistically significant.



Fig. 6. Bird anvil at site 13.4. Photo: M. OŻGO

Thrush anvils were generally rare: they occurred in only 7% of all collection sites: in 14 shaded and three open habitats. At three sites (13.4, 21.6, 22.6) predation was massive, and there were probably thousands of shells broken by the birds. Table 4 presents morph frequencies in live and predated snails collected at the same sites. Overall, there was no consistency in

the direction of differences. Only seven comparisons were significant, and they concerned different morphs and different directions of differences. The only repeatable result was that unbanded were more frequent among predated than live snails; this was however non-significant over all sampled sites (Wilcoxon paired-sample test  $p=0.065$ ).

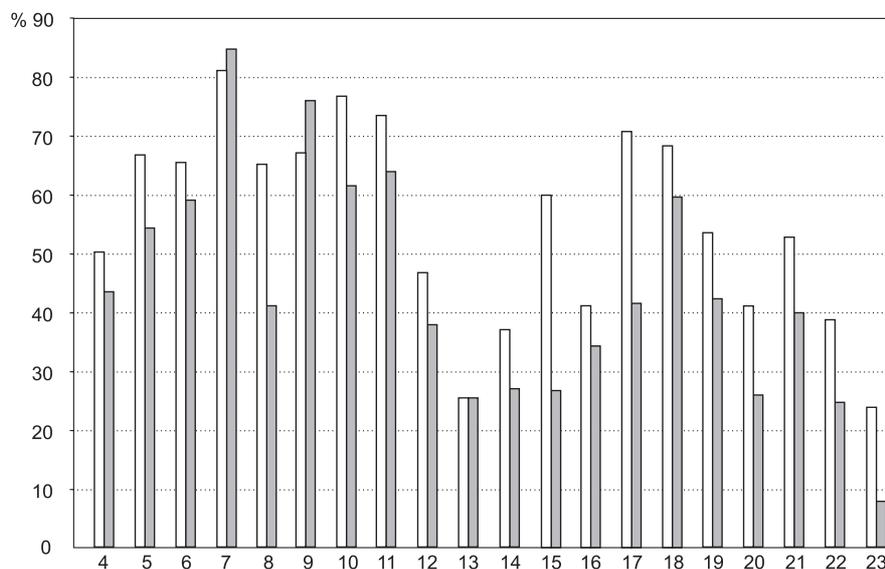


Fig. 7. Frequencies of yellow shells at open (white bars) and shaded (grey bars) habitats. 4–23 – plot numbers

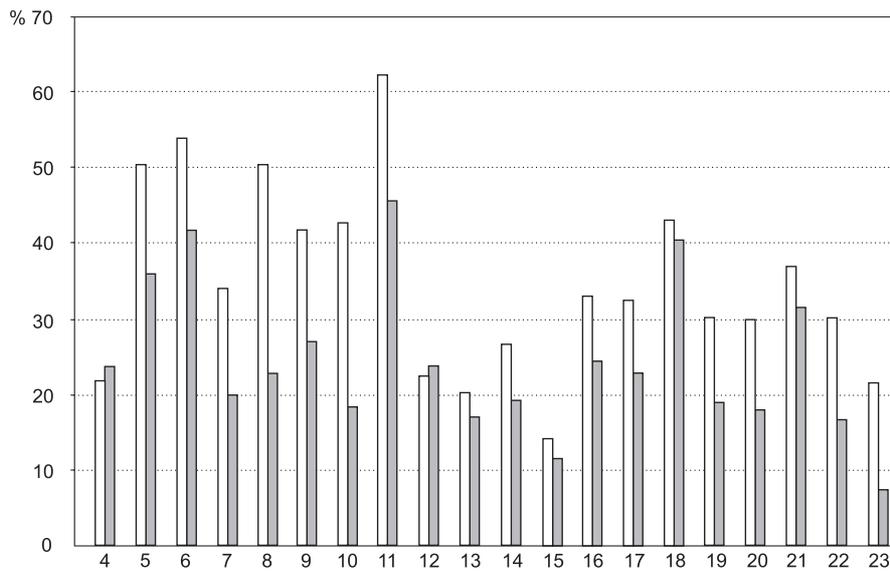


Fig. 8. Frequencies of yellow effectively unbanded shells at open (white bars) and shaded (grey bars) habitats. 4–23 – plot numbers

### DISCUSSION

Along the whole transect, the frequencies of light shells were higher in the open than in the shaded habitats. Within individual study plots, the differences in morph frequencies between habitat types were not large, amounting to approximately 10%, but throughout the study area they were consistent and highly significant. A similar difference of around 10% in the frequencies of yellow shells was found in northern Poland (CAMERON et al. 2011), while in south-eastern Poland it was higher, reaching about 30% (OŻGO 2005b). In both those areas populations of *C. nemoralis* are of a relatively recent origin, but the climate in south-eastern Poland is more extreme and thus selection is probably stronger there. The difference observed in the present study is also less than that seen

in Great Britain at the same latitude, where unlike here, unbanded shells were more frequent in shaded than in open habitats (R. A. D. CAMERON, pers. comm.). Brown shells were present in the central part of the Polish section of the transect and showed a greater frequency of occurrence in shaded habitats, as they did around Gdańsk (CAMERON et al. 2011), directly north of the transect at this point.

The morphs that showed the habitat effect in the present study: yellow and yellow effectively unbanded, suggest that it resulted from climatic rather than visual selection. Other evidence also suggests that visual selection had a very limited impact. Thrush anvils indicating bird predation and thus visual selection were rare throughout the study area. In places where they were present, the differences in morph frequencies between live and predated snails were not consistent

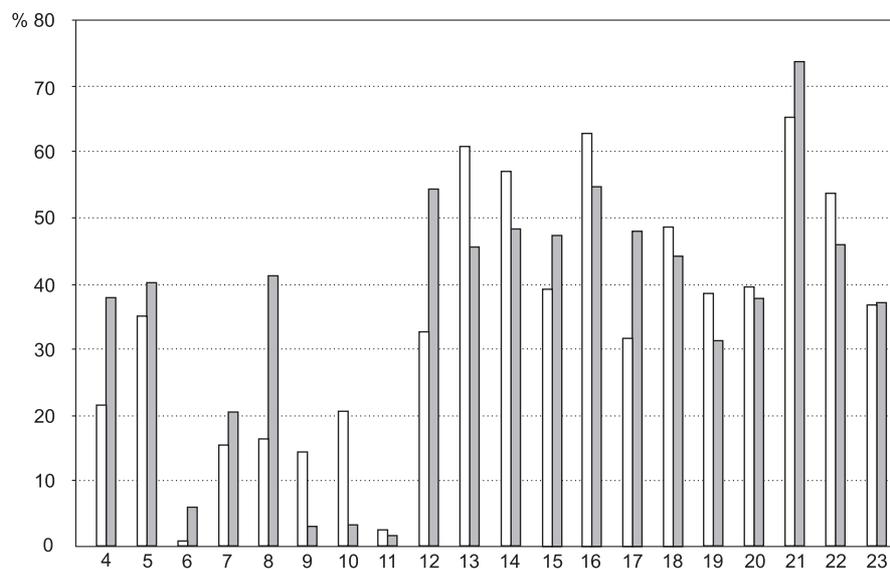


Fig. 9. Frequencies of mid-banded shells at open (white bars) and shaded (grey bars) habitats. 4–23 – plot numbers

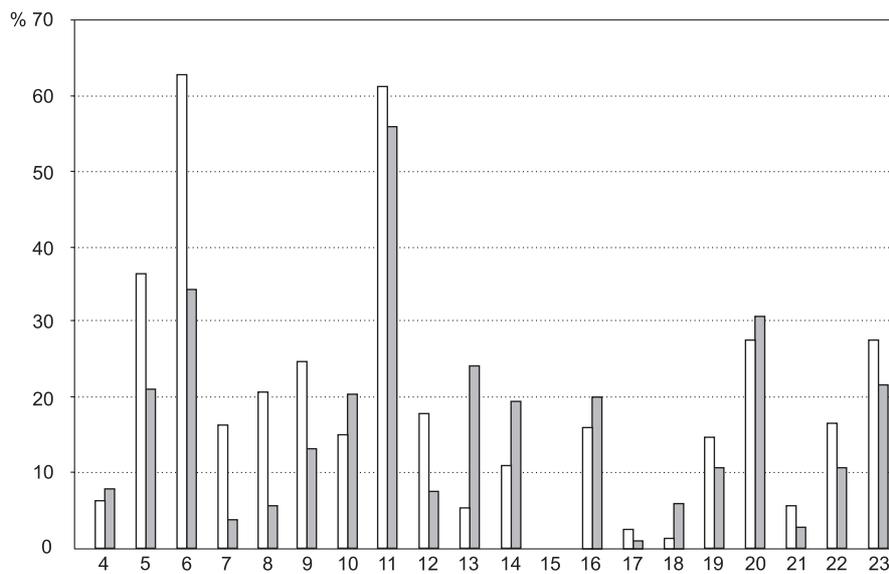


Fig. 10. Frequencies of three-banded shells at open (white bars) and shaded (grey bars) habitats. 4–23 – plot numbers

for any of the analysed phenotypes, and were mostly non-significant: out of 78 comparisons, only seven were significant (9%). There was one difference repeatable over a number of sites, i.e. the difference in the frequency of unbanded shells, but it went in the same direction in open and shaded habitats. The possible explanation is that most shaded sites did not have a uniform background, and it might very well be that banded shells were less conspicuous in both open and shaded habitats.

The method used in this study did not involve marking the snails, as had been done in the studies explicitly focused on the problem of selective bird predation (e.g. SHEPPARD 1951). Thus, it is possible that areas of human and bird collections overlapped only in part, and this could have affected the results of the comparisons. However, the prevalence of non-significant differences suggests the simplest explanation:

birds ate what was available, and they collected snails reflecting morph compositions of the populations they fed on.

The massiveness of bird predation at some places indicates that it does have the potential to exert directional selection. However, its low incidence, few significant differences in morph frequencies between live and predated snails, and direction of selection confounded between open and shaded sites indicate that selection by thrushes can probably have only local significance.

These two results: habitat effect consistent with the hypothesis of climatic selection, and lack of evidence of the effect of visual selection on morph frequencies, indicate that in the area encompassed by this study climatic selection related to the differences in microclimates between open and shaded sites was the predominant selective force affecting morph frequencies.

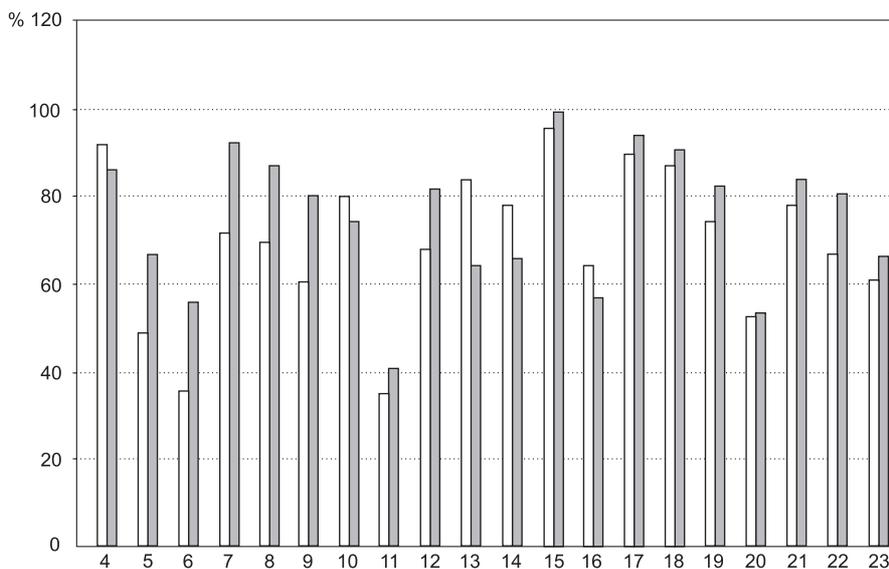


Fig. 11. Frequencies of five-banded shells at open (white bars) and shaded (grey bars) habitats. 4–23 – plot numbers



Table 4. Morph frequencies (%) among live and predated shells. N – number of specimens

Site No.	Habitat		Yellow	Unbanded	Yellow effectively unbanded	Five-banded	Band fusion	N
13.7	O	live	3.2	40.5	1.6	58.7	no data	126
		predated	4.4	34.8	0.0	33.3		23
		$\chi^2$	0.083 #	0.264 #	0.370 #	3.234 #		
15.4	O	live	59.6	7.4	3.7	85.7	25.6	136
		predated	76.2	4.8	11.9	80.0	9.4	42
		$\chi^2$	3.829 #	0.343 #	4.097 *	0.751 #	3.109 #	
20.4	O	live	26.2	24.6	20.6	20	20.6	126
		predated	38.5	23.1	38.5	0	--	13
		$\chi^2$	0.893 #	0.000 #	2.161 #	2.442 #		
9.1	S	live	64.1	7.7	7.7	83.3	63.3	39
		predated	81.8	0.0	18.2	72.7	43.8	22
		$\chi^2$	2.122 #	1.780 #	1.532 #	0.936 #	1.628 #	
9.4	S	live	72.7	23.4	25.0	72.4	27.9	128
		predated	80.0	10.0	10.0	100.0	22.2	10
		$\chi^2$	0.255 #	0.962 #	1.147 #	3.316 #	0.131 #	
12.10	S	live	49.4	37.4	22.0	57.9	no data	91
		predated	41.8	47.1	23.5	44.4		17
		$\chi^2$	0.393 #	0.567 #	0.020 #	0.571 #		
13.4	S	live	16.9	70.2	10.5	29.7	no data	124
		predated	21.9	71.7	13.9	35.0		424
		$\chi^2$	1.455 #	0.111 #	0.990 #	0.351 #		
14.1	S	live	14.0	33.9	3.3	58.8	no data	121
		predated	14.3	14.3	2.9	50.0		35
		$\chi^2$	0.001 #	5.015 *	0.018 #	0.679 #		
15.5	S	live	11.8	66.7	2.0	64.7	no data	51
		predated	9.2	64.6	1.5	60.9		65
		$\chi^2$	0.198 #	0.053 #	0.030 #	0.061 #		
15.13	S	live	51.2	38.2	38.9	21.0	35.3	131
		predated	50.8	31.8	41.3	16.4	48.1	242
		$\chi^2$	0.003 #	1.526 #	0.202 #	0.791 #	0.703 #	
16.4	S	live	71.4	11.4	48.6	32.3	no data	105
		predated	68.4	0.0	47.4	26.3		38
		$\chi^2$	0.122 #	4.741 *	0.016 #	0.449 #		
17.13	S	live	56.6	39.5	28.9	82.6	36.8	76
		predated	69.1	32.0	27.8	89.4	18.6	97
		$\chi^2$	2.872 #	1.054 #	0.026 #	1.076 #	4.001 *	
20.3	S	live	19.4	27.9	10.1	43.0	51.3	129
		predated	19.9	32.3	10.8	31.8	40.0	186
		$\chi^2$	0.013 #	0.681 #	0.037 #	2.928 #	1.013 #	
20.12	S	live	38.2	29.4	16.2	47.9	42.1	68
		predated	46.9	37.5	15.6	65.0	46.2	32
		$\chi^2$	0.671 #	0.654 #	0.005 #	1.654 #	0.051 #	
21.6	S	live	44.3	5.7	32.9	21.2	25.0	140
		predated	65.0	3.9	48.1	26.0	24.4	688
		$\chi^2$	21.016 ***	0.921 #	10.913 ***	1.349 #	0.004 #	
22.6	S	live	33.1	3.8	16.3	53.9	53.4	160
		predated	31.4	4.1	17.1	38.0	55.6	629
		$\chi^2$	0.176 #	0.044 #	0.062 #	12.916 ***	0.112 #	
22.7	S	live	3.0	59.8	0.8	88.7	34.0	132
		predated	1.1	53.3	1.1	85.7	16.7	90
		$\chi^2$	0.895 #	0.928 #	0.075 #	0.187 #	3.160 #	

Statistical significance: # P&gt;0.05; \* P&lt;0.05; \*\* P&lt;0.01; \*\*\* P&lt;0.001

In modern Europe, the occurrence of *C. nemoralis* is practically limited to anthropogenic habitats, which the snails colonise with more or less direct human help. The founder effect and genetic drift are bound to strongly affect genetic composition of many populations. Additionally, anthropogenic habitats are commonly short-lived, and undergo rapid and often dramatic changes. In view of this, the consistent habitat effect observed in this study can be interpreted as a re-

sult of an ongoing adaptation of populations of *C. nemoralis* to current environmental conditions. This confirms, on a much larger geographical scale, the earlier findings that *Cepaea* snails are capable of rapid evolutionary response to changing climatic and microclimatic conditions (CAMERON & POKRYSZKO 2008, OŹGO & KINNISON 2008, OŹGO 2011, OŹGO & BOGUICKI 2011, OŹGO & SCHILTHUIZEN 2011).

## 6. VARIATION WITHIN AND AMONG PLOTS

Apart from analyses of the environmental and geographical associations of particular morphs, the amount of variation within and among plots can be a useful indicator of both population origins and the power of any plot-wide selective forces. In this chapter, the effects of position on the transect on the range of morph frequencies are analysed.

### METHODS

While the arcsine transformed frequencies used in the ANOVA enable statistical testing, the use of estimates of  $F_{st}$  (WRIGHT 1978) is more widespread. Following CAMERON et al. (2009),  $F_{st}$  was estimated di-

rectly from morph frequencies, rather than from estimated allele frequencies (which assume Hardy-Weinberg equilibrium). Hence it was used here simply as an estimate of variance as a proportion of the maximum possible given the mean morph frequency. Estimates of  $F_{st}$  were corrected for sample size to remove sampling error; these corrections have only trivial effects. The results were compared with those from arcsine transformation.

### RESULTS

The ANOVA presented in Table 2 shows that variation among plots contributed a significant amount to

Table 5. Values of  $F_{st}$  estimated from morph frequencies for each morph. A – in each plot, B – among plots and overall. The percentage accounted for by among plot variation is also given. There were no shells with more than one band in plot 15

A	Plot	Yellow	Unbanded	Mid-banded	Three-banded	mean
	4	0.100	0.020	0.065	0.130	0.079
	5	0.111	0.177	0.148	0.288	0.181
	6	0.163	0.159	0.182	0.152	0.164
	7	0.149	0.302	0.029	0.150	0.157
	8	0.266	0.273	0.179	0.388	0.277
	9	0.321	0.249	0.242	0.262	0.269
	10	0.115	0.198	0.170	0.213	0.174
	11	0.086	0.136	0.038	0.210	0.118
	12	0.059	0.073	0.316	0.138	0.147
	13	0.056	0.183	0.303	0.404	0.237
	14	0.264	0.087	0.211	0.410	0.243
	15	0.298	0.295	0.573		0.389
	16	0.290	0.129	0.197	0.260	0.219
	17	0.350	0.334	0.367	0.170	0.305
	18	0.120	0.238	0.307	0.408	0.268
	19	0.150	0.405	0.309	0.349	0.303
	20	0.172	0.077	0.161	0.213	0.156
	21	0.021	0.166	0.063	0.197	0.112
	22	0.212	0.421	0.473	0.539	0.411
	23	0.328	0.321	0.261	0.351	0.315
B	among plots	0.130	0.083	0.157	0.141	0.128
	overall	0.256	0.249	0.348	0.363	0.304
	% due to plots	50.785	33.420	45.272	39.009	42.122



total variation, but also that variation within plots contributed more to the total. Table 5 shows the values of  $F_{st}$  by morph, overall, and by plot. Overall estimates did not differ greatly among morphs. Estimates within plots varied greatly, from less than 0.02 (i.e. variation between samples is less than 2% of that possible for the mean frequency) to 0.57 (57% of the maximum possible). Although the proportion of all variation due to differences among plots differed between the two methods of estimation, they showed comparable trends (see Table 2), with the proportion of all variation occurring within plots being greatest for unbanded. For both  $F_{st}$  and arcsine-transformed frequencies, variances in all morphs were positively associated (Table 6), although those between yellow and the banding morphs failed to reach significance. Overall variability along the cline showed the same pattern with both estimates (Fig. 12). For the correlation between the two measures,  $r=0.968$ ,  $P<0.001$ . There was a pattern of peaks and troughs that did not relate to climatic variables.

## DISCUSSION

Given the length of the transect, overall values of  $F_{st}$  were not very high, though they fell into the cate-

Table 6. Correlations between within plot values of  $F_{st}$  and arcsine transformed variances for each major morph

Comparison	$F_{st}$		Arcsine	
	r	P	r	P
Y and U	0.420	ns	0.413	ns
Y and M	0.351	ns	0.428	ns
Y and T	0.084	ns	0.233	ns
U and M	0.666	<0.01	0.522	<0.05
U and T	0.448	<0.05	0.481	<0.05
M and T	0.482	<0.05	0.584	<0.01

Y – yellow, U – unbanded, M – mid-banded, T – three-banded

gory of very great genetic differentiation as defined by WRIGHT (1978). Within-plot variation accounted for a substantial proportion of the total. In some cases, it reached levels similar to those seen in “area effects” (CAIN & CURREY 1963; see comparative data in CAMELON et al. 2009), indicating very strong patterns of local differentiation. In others, the low levels corresponded to those in areas in which there were large, stable and well-connected populations subject to similar environmental conditions. The trend for values for different morphs to co-vary is considered in Chapters 9 and 10.

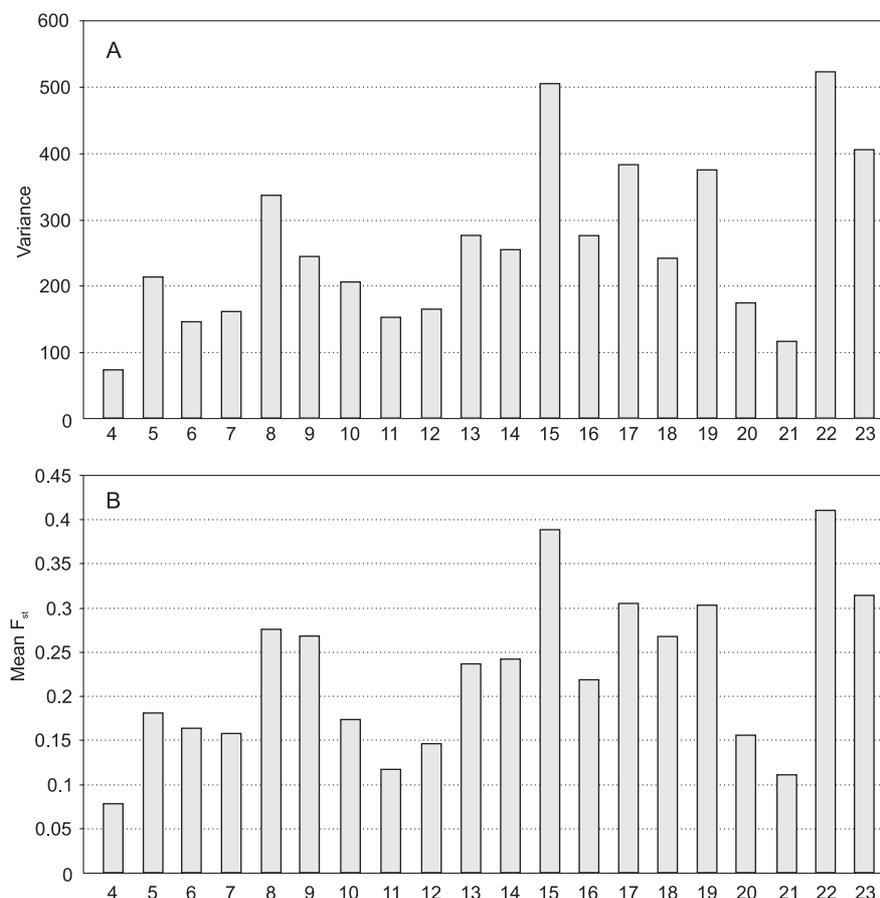


Fig. 12. Variation in mean variance of morph frequencies among plots: A – based on arcsine transformed frequencies, B – based on estimates of  $F_{st}$

## 7. FUSION OF BANDS

Variation in the fusion of bands is one of the most enigmatic elements of shell polymorphism in *Cepaea* snails. The mode of inheritance is less clear than that of other shell characteristics, but most probably it does have a genetic basis (CAIN et al. 1960, WOLDA 1969). Although band fusion has been studied less intensively than variation in shell colour and banding, there are indications that it does respond to climatic and/or visual selection. In *C. vindobonensis*, in which the ground colour of the shell is always yellowish-white and the number of bands is usually five, it is the fusion of bands that responds to climatic selection (ROTARIDES 1926, OŹGO & KOMOROWSKA 2009). In

*C. hortensis* fusion of bands was observed to be more frequent in woods and under ivy than in hedgerows and rough herbage (CLARKE 1960). In populations of *C. nemoralis* variation in the ground colour of the shells is usually larger than in either of those species, which makes the study of the fusion of bands more complicated. Interestingly however, in populations introduced in Lynchburg, Virginia (USA) in which only yellow-shelled snails occurred, fusion of bands clearly responded to habitat shadiness (RICHARDS & MURRAY 1975). CAMERON & PANNETT (1985) found a higher frequency of fusions in woodland populations of *C. nemoralis* than in the open, and detected this trend

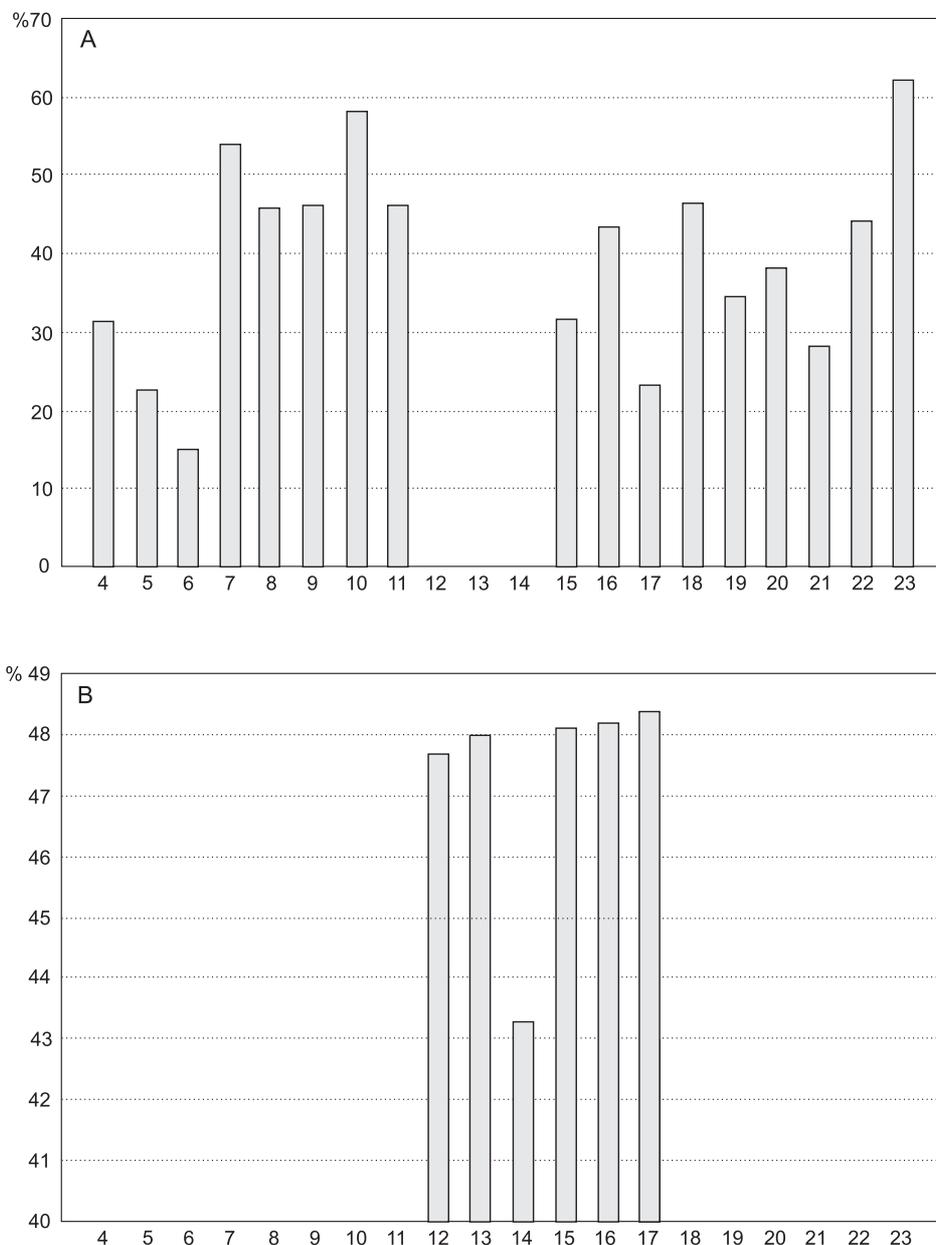


Fig. 13. Variation in the fusion of bands along the transect: A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text

even in recently created woods. In human-impacted unstable habitats, in which adaptation of *C. nemoralis* populations can occur on contemporary time-scales, fusion of bands is one of the features responding rapidly to habitat changes (OŽGO 2011).

In this chapter variation in the fusion of bands is analysed in relation to large-scale climatic conditions and to habitat shadiness.

#### MATERIAL AND METHODS

All five-banded shells were scored for the fusion of bands. There are basically two methods to do that.

**Method 1.** This method determines whether two or more individual bands are joined together and form

one broader band. This is scored at a line across the body-whorl at the right angle to the lower lip of the mouth (CAIN & SHEPPARD 1950). Band fusion is indicated by parentheses around the band digits that are joined. For example, 123(45) indicates that bands 4 and 5 are fused together, and (12345) indicates that all five bands are joined. This method of scoring is relatively simple and quick, but involves a certain degree of subjectivism and is not always repeatable, even when the same person scores the shells. Additionally, there is a lot of variation in the width of individual bands, which can range from very narrow to quite broad, even if not exactly joined; a yellow five-banded shell with unfused bands can in fact be quite light when all bands are narrow, or dark when banding is heavy.

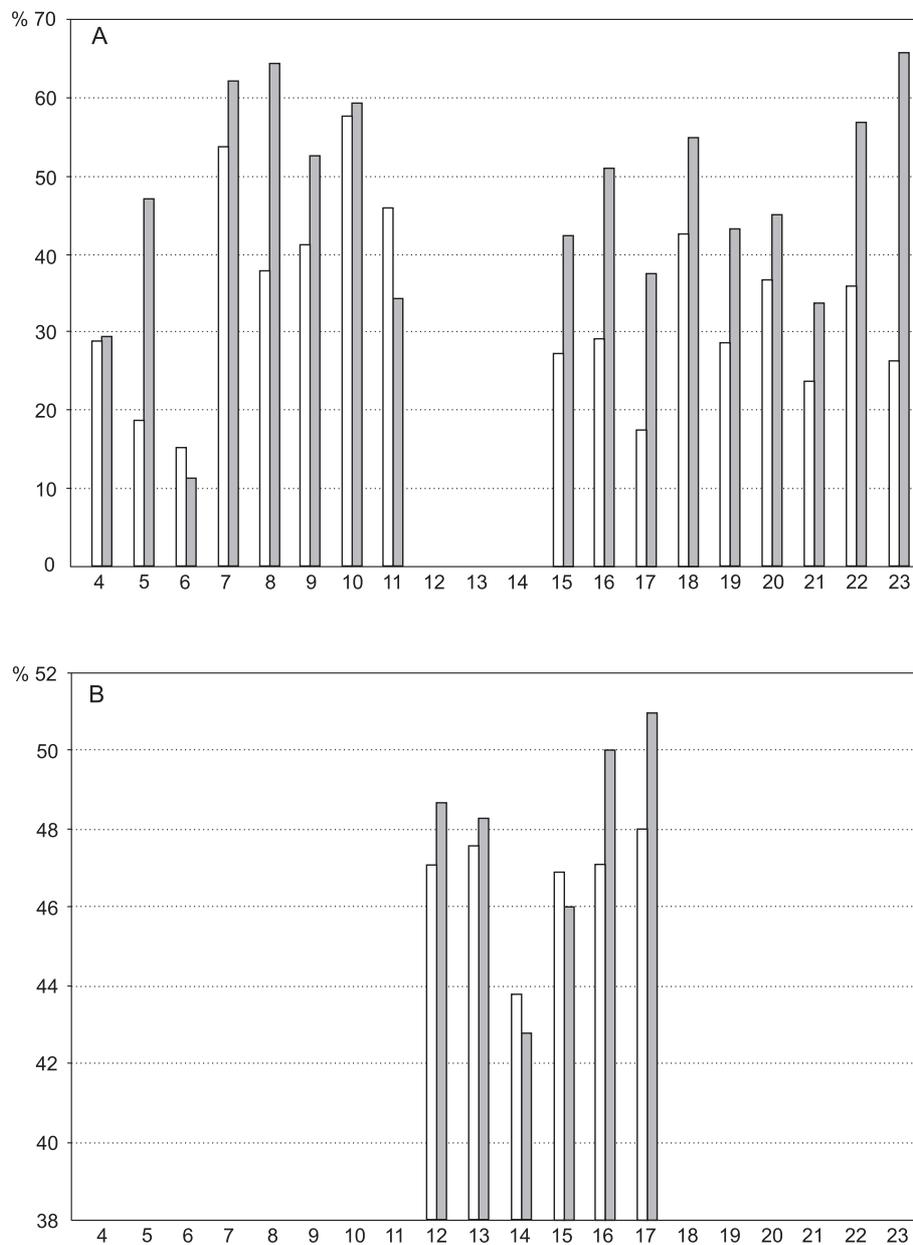


Fig. 14. Variation in the fusion of bands in yellow (white bars) and in pink shells (grey bars): A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text

**Method 2.** This method involves measuring the width of all the bands and of the ground area, and calculating the proportion of the shell surface covered with bands (%B). This, as in Method 1, is done at a line across the body-whorl at the right angle to the lower lip of the mouth. The measurements are done through a magnifying glass, with the accuracy of 0.5 mm (ROTARIDES 1926, OŻGO & KOMOROWSKA 2008). This method is more objective and allows to account for the differences between lightly and heavily banded shells, even when the bands are not joined. Hence, it might be regarded as a measure of band coverage, and is best summarised as the mean proportion of shell covered by bands. However, it is much more time-consuming, and often impractical in field conditions.

In the first two years of data collection, i.e. at plots 12–17, method 2 was used, so in these plots the width of the bands as a proportion of the shell whorl is used as a measurement of band fusion. In subsequent years fusion of bands was scored with method 1. As these methods basically differ only in accuracy, they can be expected to give consistent results. For simplicity, the results obtained with both methods are further referred to as “fusion of bands”.

Significance of differences in comparisons within the whole data set was determined with the Mann-Whitney test; in paired-sample comparisons the Wilcoxon test was used.

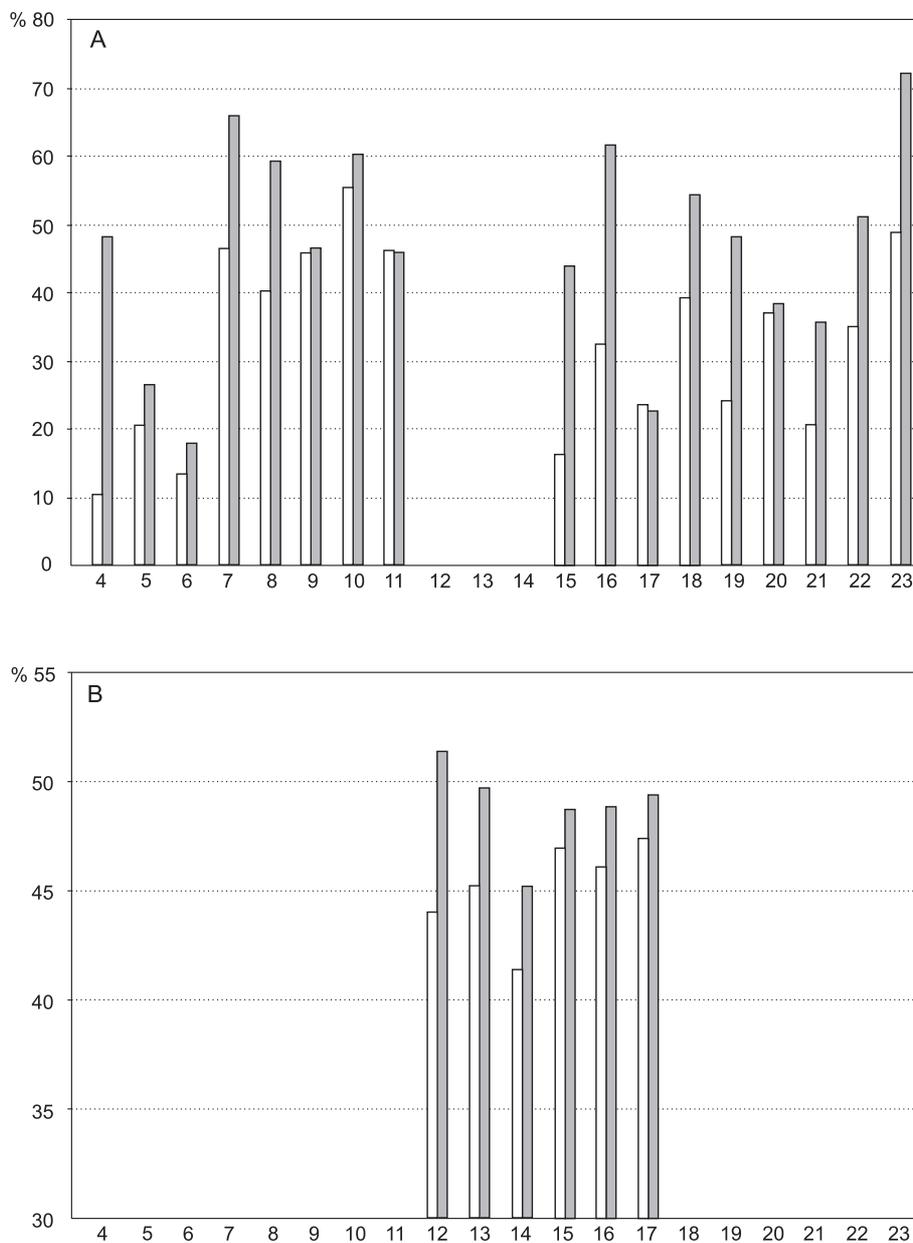


Fig. 15. Variation in the fusion of bands in open (white bars) and in shaded habitats (grey bars): A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text



RESULTS

In plots 4–11 and 15–23 fusion of bands was scored with method 1, in plots 12–17, with method 2 (in plots 15–17 both methods were used, but in different samples). Figures 13A–19A present frequencies of band fusions in plots 4–11 and 15–23, and Figures 13B–19B present average proportions of shell surface covered with bands in plots 12–17. Full data are given in Appendices 4 and 5.

The following patterns are evident:

1. Variation in the fusion of bands along the transect was large, idiosyncratic and not correlated with any large-scale climatic parameters (Fig. 13).
2. Generally, fusion of bands was higher in pink than in yellow shells (Fig. 14). Plots 4–11 and 15–23: Mann-Whitney test  $p=0.006$ , Wilcoxon test  $p=0.002$ . Plots 12–17, the difference was not significant.
3. In open habitats fusion of bands was significantly lower than in shaded habitats (Fig. 15). Plots 4–11 and 15–23: Mann-Whitney test  $p=0.009$ , Wilcoxon test  $p=0.0006$ . Plots 12–17: Mann-Whitney test  $p=0.020$ , Wilcoxon test  $p=0.028$ .
4. In open habitats fusion of bands was lower in yellow than in pink shells (Fig. 16). Plots 4–11 and 15–23: Mann-Whitney test  $p=0.0006$ , Wilcoxon test  $p=0.00002$ . Plots 12–17: Mann-Whitney test

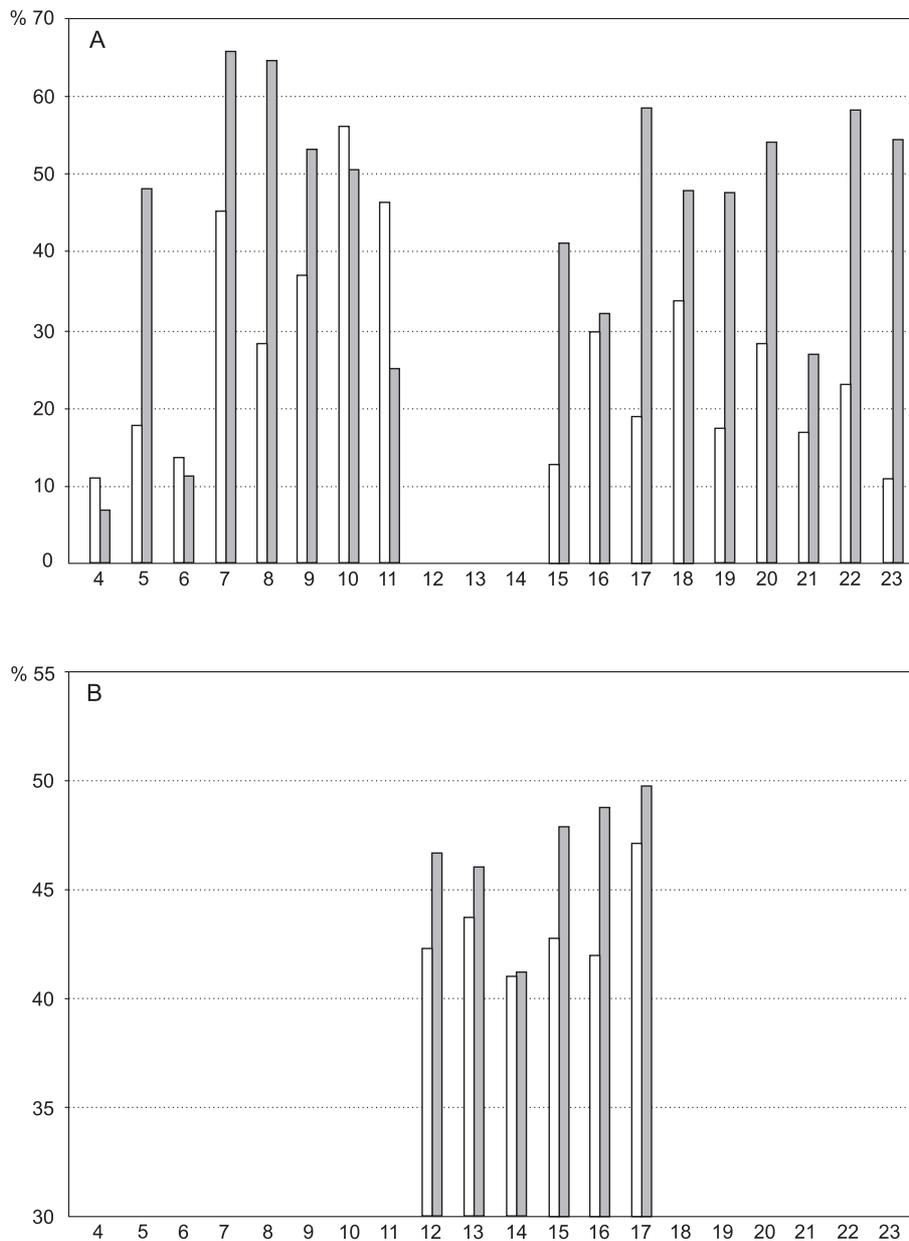


Fig. 16. Variation in the fusion of bands in yellow (white bars) and pink shells (grey bars) in open habitats: A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text

$p=0.078$ , Wilcoxon test  $p=0.028$ . In shaded habitats the differences between yellow and pink shells were much smaller (Fig. 17) and significant only in plots 4–11 and 15–23; Mann-Whitney test  $p=0.016$ , Wilcoxon test  $p=0.0006$ .

5. In yellow shells fusion of bands was lower in open than in shaded habitats (Fig. 18). Plots 4–11 and 15–23: Mann-Whitney test  $p=0.007$ , Wilcoxon test  $p=0.001$ . Plots 12–17: Mann-Whitney test  $p=0.004$ , Wilcoxon test  $p=0.028$ . In pink shells there was no difference in band fusion between habitat types (Fig. 19).

## DISCUSSION

Variation in the fusion of bands did not show any discernible association with large-scale climatic conditions. Some other patterns were however evident. Overall, fusion of bands was more frequent in pink than in yellow shells. Higher frequencies of band fusion in pink than in yellow shells were also observed in Spain (RAMOS 1985). Possibly this is a more universal pattern, but I am not aware of any other studies dealing with this.

Band fusion was significantly higher in shaded than in open sites, consistently with the climatic selection hypothesis. Interestingly however, this re-

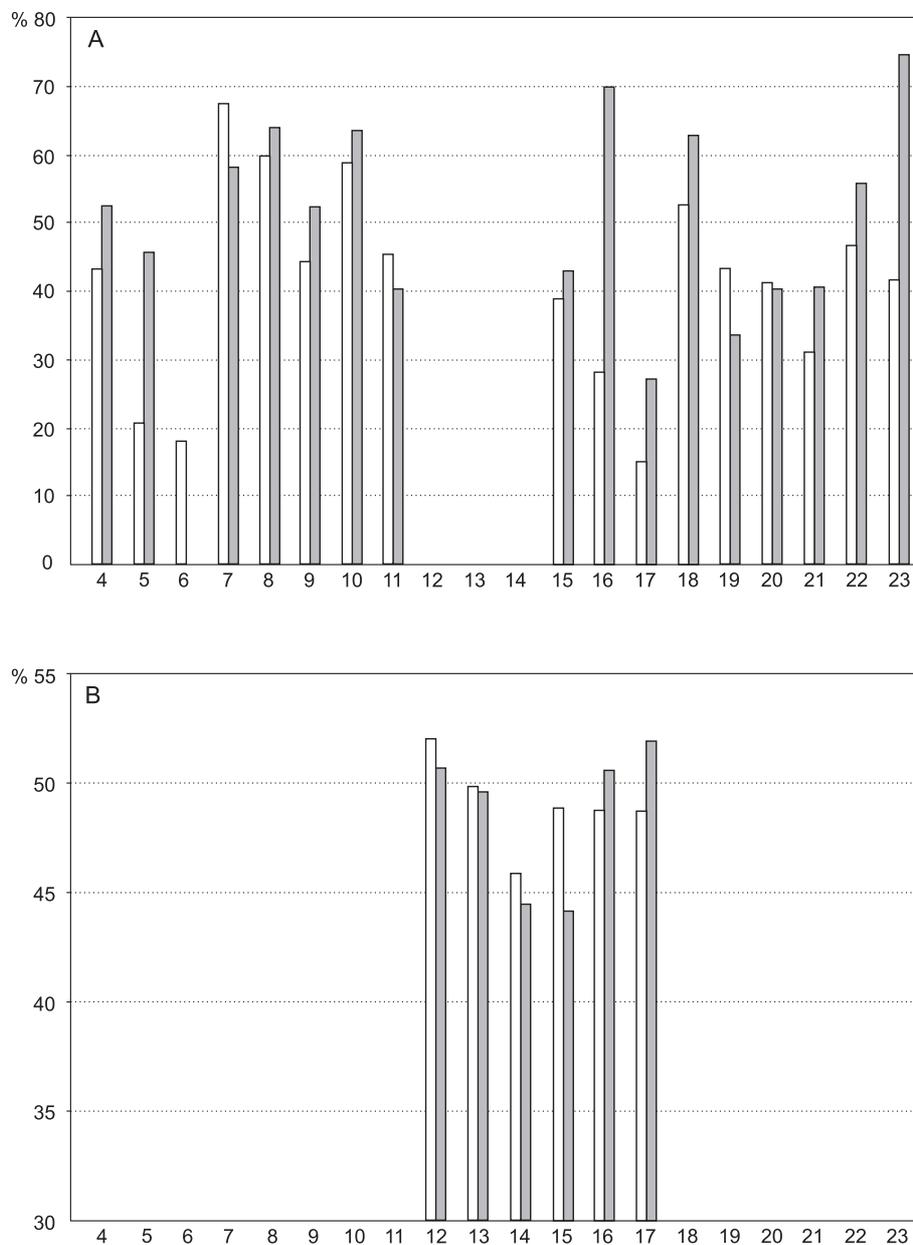


Fig. 17. Variation in the fusion of bands in yellow (white bars) and pink shells (grey bars) in shaded habitats: A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text



sponse was observed only in yellow shells, in which there was a significant difference in band fusion between habitat types; pink shells showed no such response. The possible explanation is that, as pink shells are generally dark, differences in thermal properties between pink shells with separate and with fused bands are not large. In contrast, thermal properties of yellow shells with narrow, separate bands can differ markedly from thermal properties of yellow shells with broad, fused bands. An additional interesting aspect is that the difference in band fusion between yellow and pink shells was observed only in open habitats, while in shaded places

yellow five banded shells were as fused as pink five-banded. This might indicate that selection operates more strongly, and is therefore more diversifying in open than in shaded habitats.

In summary, fusion of bands showed response to habitat shadiness consistent with the hypothesis of climatic selection, but it was evident only in yellow shells. This means that the effects of climatic selection on band fusion can be expected to be statistically detectable only in populations with high frequencies of yellow five-banded snails. This possibly explains why variation in band fusion often appears to behave erratically.

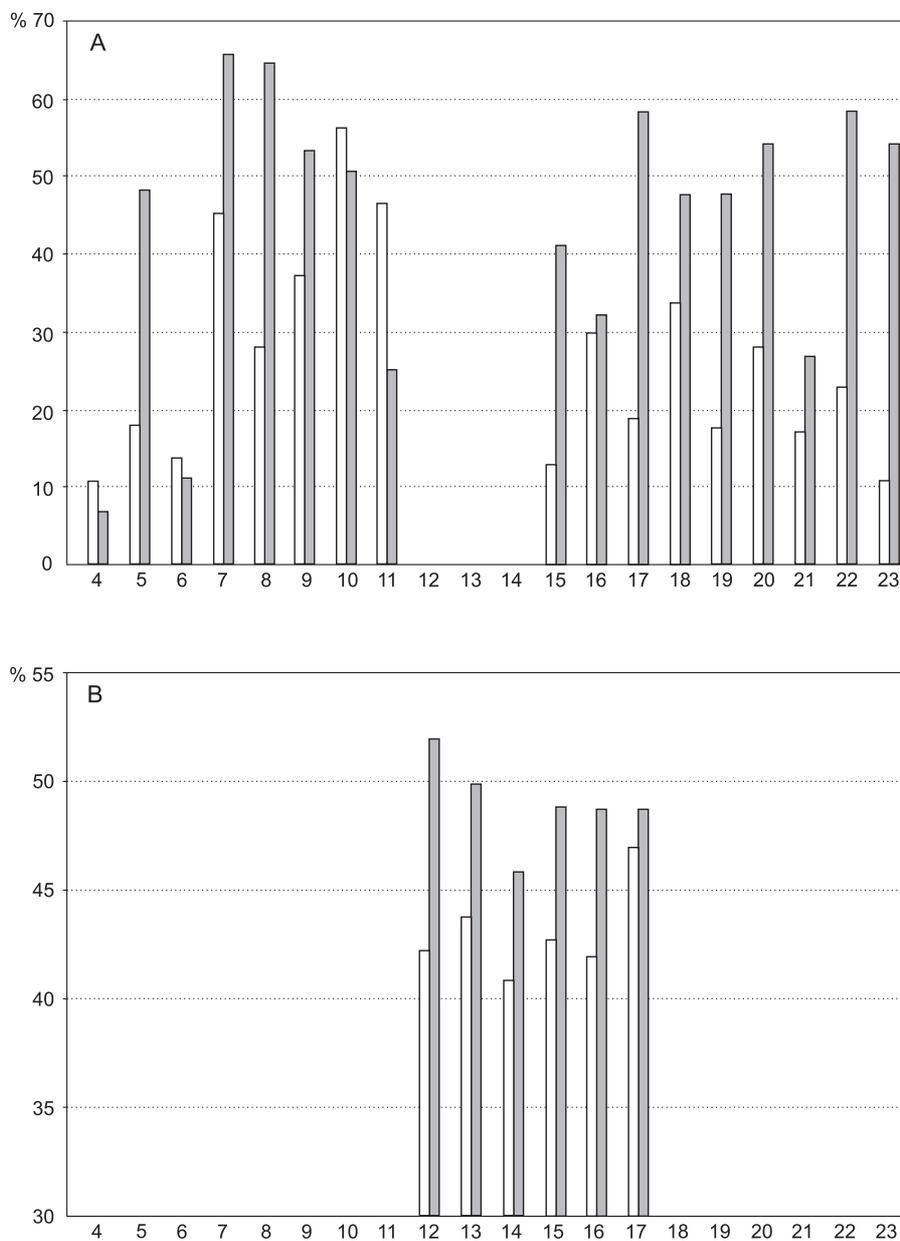


Fig. 18. Variation in the fusion of bands in yellow shells in open (white bars) and shaded habitats (grey bars): A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text

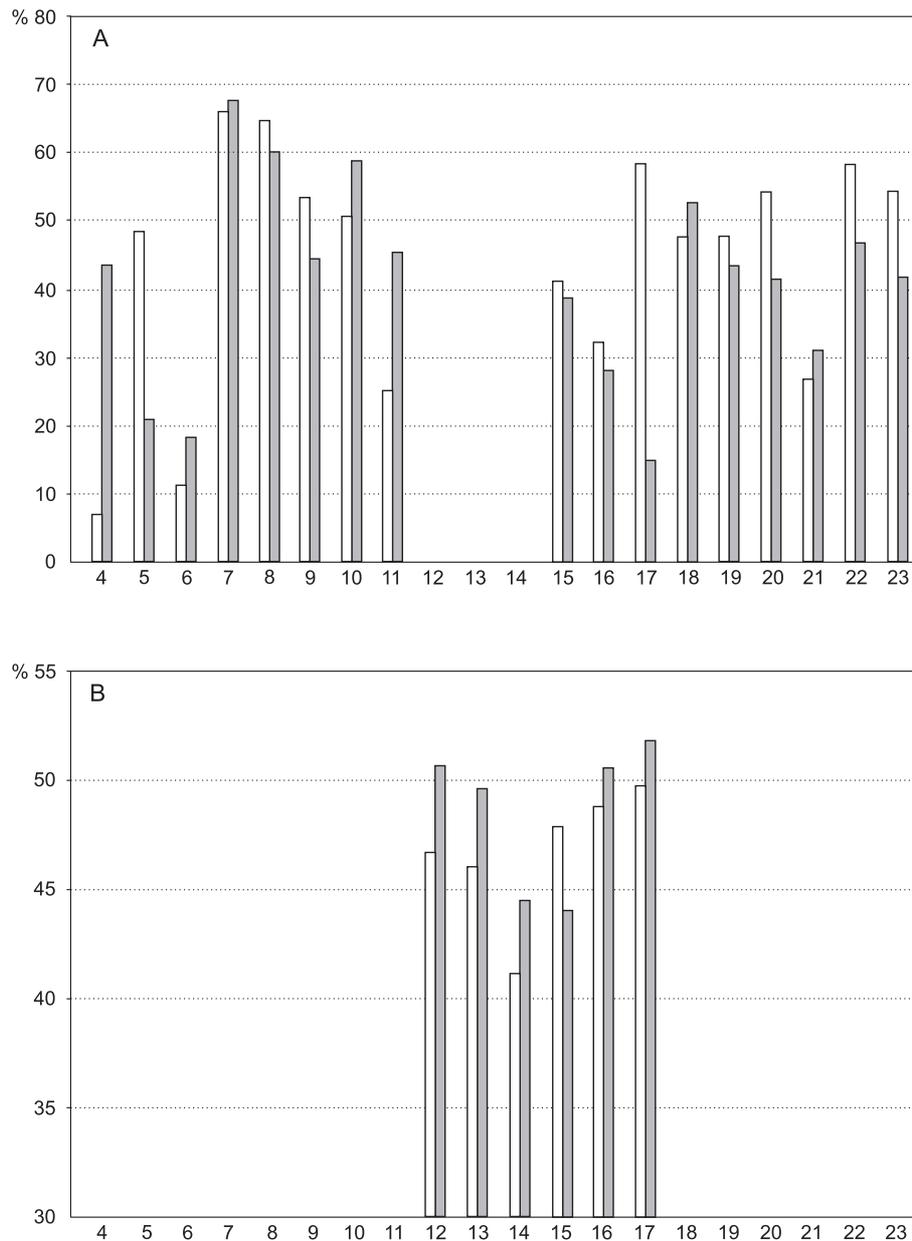


Fig. 19. Variation in the fusion of bands in pink shells in open (white bars) and shaded habitats (grey bars): A – scored with method 1; B – scored with method 2. 4–23 – plot numbers, for methods – see text

## 8. THE LEVEL OF POLYMORPHISM

Genetic variation can be expected to be lower at the edge of the distribution of a species than in more central populations (SOULÉ 1973). One of the reasons is that while in central, optimal environments, stabilising selection will be the dominant mode, in marginal environments directional selection will prevail, and in effect, attrition of genetic variation may occur (SOULÉ 1973). Isolation and small size of peripheral populations may also increase the chance of founder effects, causing a loss of genetic diversity. In a polymorphic species the degree of polymorphism can serve as a measure of genetic variation. Towards the geographic

limit of distribution of a species, reduction in the degree of polymorphism can be expected; within the same geographic region, a lower level of polymorphism can be expected in more extreme environments where directional selection is stronger. In south-eastern Poland, populations of *C. nemoralis* inhabiting open habitats were significantly less polymorphic than populations in shaded habitats. This suggested that in more exposed habitats, characterised by more extreme climatic conditions, selection pressure acted against certain genotypes and resulted in a tendency towards monomorphism (OŻGO 2005b). A similar result was



obtained by BANTOCK & PRICE (1975) on the Brendon Hills, England, where *C. nemoralis* was more polymorphic in woodlands than in open areas.

In this chapter the level of polymorphism along the transect is analysed in view of these two hypotheses: that the level of polymorphism decreases towards the edge of geographic range of the species, and that it is lower in exposed habitats, where stronger directional selection can be expected.

## METHODS

Simpson's index of polymorphism is a measure of the degree of diversity in populations of polymorphic species. It gives the probability that two individuals chosen at random from a population are of different morphs; the higher its value, the more even is the distribution of morphs in a population (SIMPSON 1949). Index of polymorphism is calculated according to the formula:

$$IP = 1 - \sum_{i=1}^m p_i^2$$

where  $p_i$  is the frequency of the  $i^{\text{th}}$  morph, and  $m$  is the number of morphs. Here, twelve basic morphs (see Chapter 2) were included. The median value of the polymorphism index was calculated for each plot, and for open and shaded habitats within the plots.

## RESULTS

Figure 20 presents variation in the values of the polymorphism index along the transect, and Table 7 gives its median values at open and shaded sites.

Table 7. Median values of the index of polymorphism at open and shaded sites

Plot	Open	Shaded
4	0.776	0.791
5	0.716	0.792
6	0.717	0.664
7	0.707	0.531
8	0.753	0.757
9	0.691	0.691
10	0.702	0.723
11	0.636	0.766
12	0.745	0.770
13	0.732	0.724
14	0.772	0.744
15	0.620	0.674
16	0.698	0.780
17	0.612	0.701
18	0.729	0.763
19	0.686	0.693
20	0.840	0.817
21	0.765	0.697
22	0.708	0.643
23	0.643	0.616

Changes in the values of the index did not coincide with any large-scale climatic variables. There was no decrease in the level of polymorphism towards the limits of the species' range, and along the whole transect, differences between open and shaded habi-

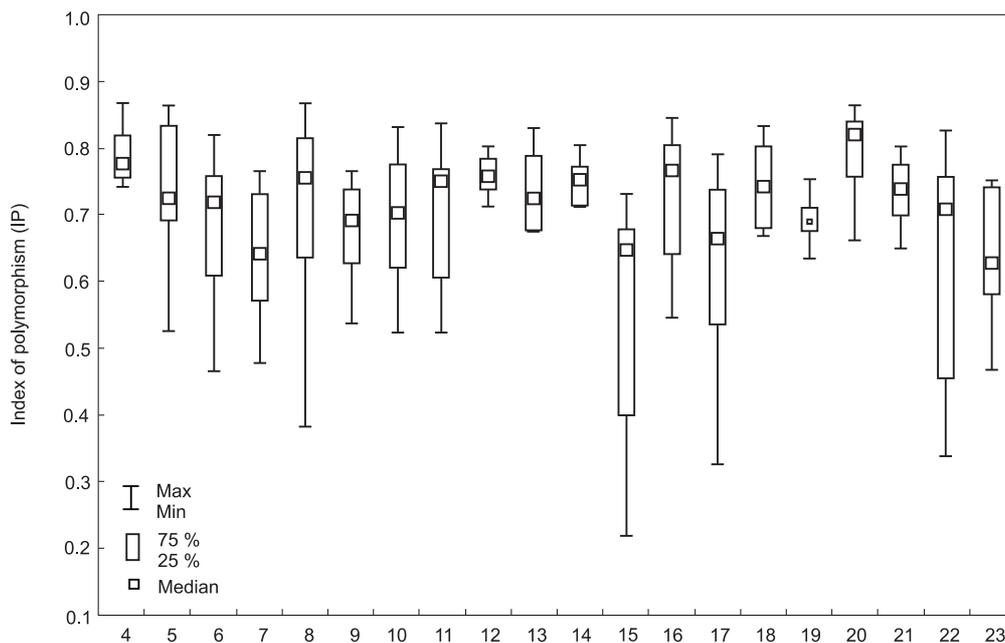


Fig. 20. Variation in the values of the index of polymorphism along the transect (max and min without outliers). 4-23 - plot numbers

tats were not consistent in direction (Wilcoxon test  $p=0.629$ ). Only in the middle part of the transect, in plots 8–19 (with the exception of plots 13 and 14), polymorphism was higher in shaded than in open habitats (Wilcoxon test,  $p=0.038$ ).

## DISCUSSION

Nearly all populations in this study had moderate to high levels of polymorphism. No decrease in the level of polymorphism was observed towards the limits of the geographic range of *C. nemoralis*, even though such decrease might be expected on theoretical grounds (SOULÉ 1973). This is explainable in view of some characteristics of the species. The mode of dispersal in this species is often leptokurtic (IBRAHIM et al. 1996), i.e. long distance migration is undertaken by a small number of individuals, and is followed by subsequent local migration. While this might, through founder effects, reduce the range of genetic variation, there are aspects of the biology of the species that reduce such effects. The mating system buffers populations of this species from drastic changes in gene frequency (MURRAY 1964). *C. nemoralis* is a hermaphroditic obligatory outcrosser. The snails mate several times during the season and mating is random with regard to colour morph (WOLDA 1963). The sperm is stored, sometimes for more than one year, so that when eggs come to be laid they may be fertilised by sperm from several matings (JONES et al. 1977). Even in an extreme case of a population being founded by a single fertilised individual, the gene pool will consist of an average of six haploid sets of genes. This system protects populations of *C. nemoralis* from the impoverishment of the gene pool (MURRAY 1964). Additionally, in a highly polymorphic species similar

directional selection may result in a variety of genetic responses: there are many possible ways of being dark or light, and the specific route of adaptation depends on the initial composition of the population (OŻGO 2011). When these features are combined, high and relatively uniform level of polymorphism throughout the species range is to be expected.

Important in the explanation of no apparent decrease of genetic variation towards the limits of the distribution of the species is the fact that the current limits of the distribution of the species in continental Europe are not really marginal. In the west, the occurrence of the species ends abruptly for the obvious reason of there being no more land to inhabit. This is not a range limit in the ecological sense, and the species inhabits the British Isles farther to the west. In the east, *C. nemoralis* is dynamically expanding its range. As the current margin of distribution is dispersal-rather than climate-limited (OŻGO & BOGUCKI 2011), the eastern populations most probably do not face considerably higher environmental stress than the more central ones. Rapid range expansion is in fact an evidence against the assumption of ecological marginality (SOULÉ 1973).

Along the whole transect, there was no consistency in the direction of the differences between open and shaded habitats. Thus the hypothesis that directional selection – presumably stronger in open than in shaded habitats – operates towards diminishing the level of polymorphism is not borne out. However, over a relatively large part of the studied area, the level of polymorphism was lower in open than in shaded habitats. This is in accordance with the expectations, and suggests that this issue requires further studies in other parts of the range of the species.

## 9. PHENOTYPES ENCODED BY UNLINKED LOCI: BANDING VARIATION IN RELATION TO SHELL COLOUR

The knowledge of the mode of inheritance of shell characteristics in *C. nemoralis* provides an exceptional opportunity to gain insight into the ways in which genetic architecture affects the routes of adaptation in natural populations. In this species the loci controlling the ground colour of the shell and the presence or absence of bands are tightly linked, while the loci that control the number of bands on a banded shell are unlinked to colour and to each other (MURRAY 1975). Linkage disequilibria have been discussed in a number of papers (GUERRUCCI-HENRION 1966, WAGNER 1990, COOK 2005), and in this study are analysed in Chapter 10. The present chapter deals with relative frequencies of phenotypes encoded by unlinked loci.

## METHODS

Frequencies of the major banding forms: mid-banded, three-banded and five-banded, were calculated as a proportion of shells capable of showing banding. In the present analysis, only populations with ten or more banded shells in both yellow and pink were included. Although the hierarchy of dominance would be more fully reflected if the three-banded and five-banded were calculated within many-banded shells, in large parts of the study area such shells were not numerous enough to allow a meaningful analysis.

Co-variation in banding forms between yellow and pink shells was determined with the Pearson coefficient of correlation.



RESULTS

Along the transect, the frequencies of the major banding forms were very similar in yellow and pink shells (Fig. 21); the correlation within samples was significant in the majority of the plots (Table 8), even though the number of samples that could be included was small. As a rule, when unbanded shells were ex-

cluded, there was a very high accordance in the frequencies of banding morphs between colours.

DISCUSSION

The present results show that in a vast majority of populations the frequencies of mid-banded, three-banded and five-banded calculated as the pro-

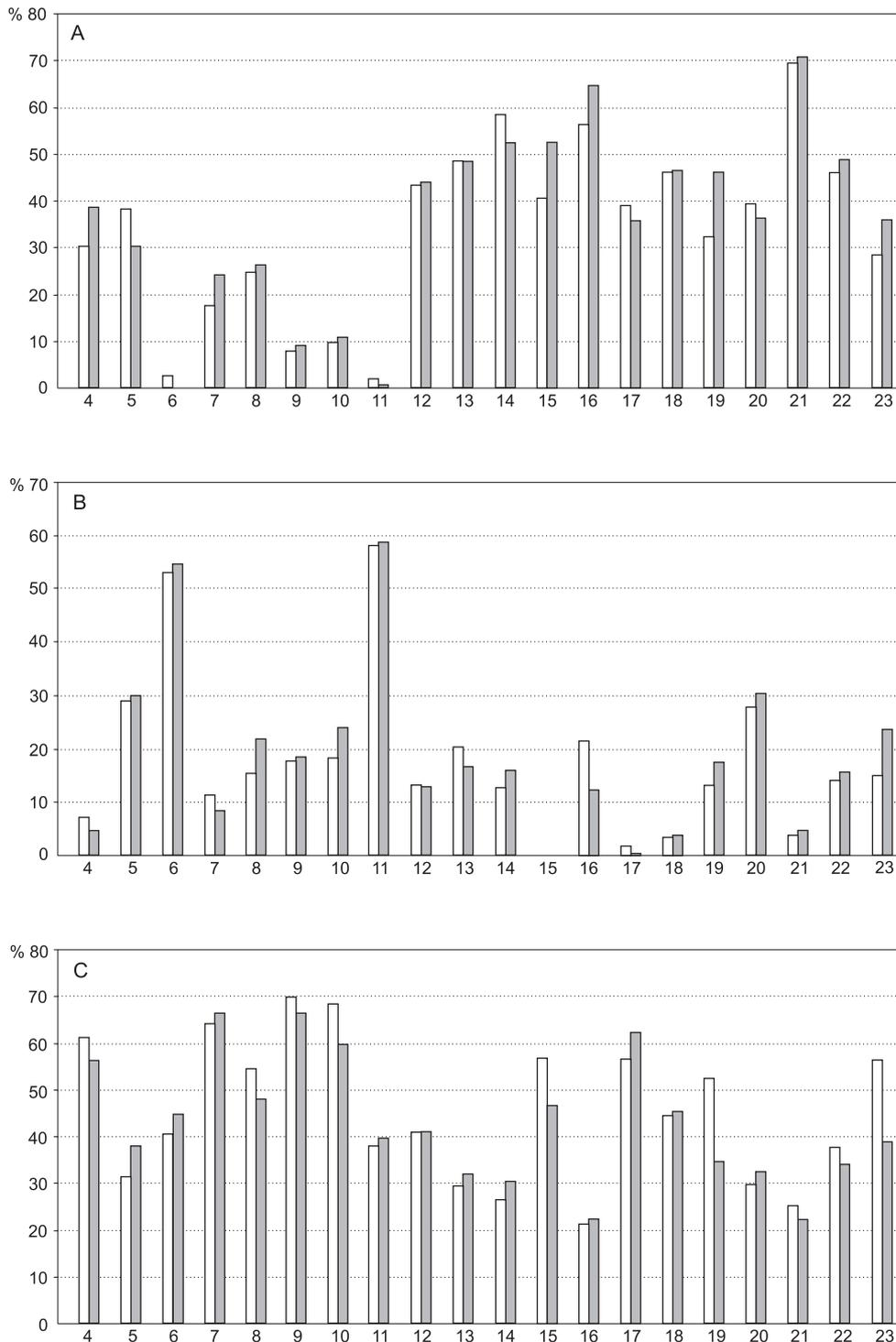


Fig. 21. Variation in the frequencies of the major banding forms in yellow (white bars) and pink shells (grey bars): A – mid-banded; B – three-banded; C – five-banded. 4–23 – plot numbers

Table 8. Pearson's coefficient of correlation of the frequencies of banding morphs between yellow and pink shells

Meridian	Mid-banded	Three-banded	Five-banded	d.f.	N
4	0.552 #	0.559 #	0.821 **	7	9
5	0.733 *	0.900 **	0.123 #	6	8
8	0.697 #	0.815 *	0.741 #	5	7
10	0.845 **	0.866 **	0.902 ***	7	9
11	0.989 ***	0.840 *	0.830 *	4	6
12	0.941 ***	0.768 **	0.909 ***	8	10
13	0.942 ***	0.986 ***	0.854 **	6	8
14	0.915 **	0.970 ***	0.924 **	6	8
15	0.976 ***	–	0.984 ***	6	8
16	0.879 ***	0.779 **	0.889 ***	9	11
17	0.958 ***	0.959 ***	0.956 ***	6	8
18	0.948 ***	0.994 ***	0.922 ***	12	14
19	0.941 **	0.990 ***	0.706 #	5	7
20	0.895 ***	0.916 ***	0.584 #	9	11
21	0.771 **	0.876 ***	0.906 ***	8	10
22	0.973 ***	0.988 ***	0.979 ***	10	12

Statistical significance: #  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

portion of banded shells were very similar in yellow and pink shells. This contrasts with the distribution of unbanded shells between colours (see Chapter 10), and results from the genetics of the species. While the loci for the ground colour and the presence or absence of bands are tightly linked, the loci determining the number of bands on a banded shell are independent from colour and from each other. In a panmictic population, even when initially there is no equilibrium, segregation of alleles will eventually even out the proportions of banding forms between colours.

In the area encompassed by this study, the populations inhabited mainly unstable habitats, where recent introductions to novel localities and frequent reintroductions can be presumed to occur commonly. In this view, the strong accordance of banding frequencies between colours is rather striking. Unfortunately, because of generally low frequencies of banded shells in plots 7–9, the present data do not allow to determine if such equilibria exist also in areas where the species inhabits the most heavily human-impacted countryside, and where the populations may be exceptionally short-lived. Nevertheless, the habitats in the whole study area were far from ancient, and almost all populations can be assumed to

be rather short-lived, so that equilibria between colour and banding within banded shells must have been reached within relatively short time. This is in accordance with an observation of an experimentally introduced population where only 11 years, i.e. three to four snail generations, were sufficient for considerable reshuffling of genetic material to occur, so that banding frequencies between colours reached almost equal proportions (OŻGO & BOGUCKI 2011).

The universally occurring accordance in the frequencies of banding morphs between colours can have its practical application. Sometimes we need to check the reliability of sampling done by other people, for example in citizen-science projects (e.g. SILVERTOWN et al. 2011) or in supervising the work of the students. The pattern is not immediately obvious: in most populations the frequencies of unbanded shells differ significantly between colours, so – when calculated as proportions of all shells – frequencies of various banding forms are usually quite different in pink and yellow shells. High correlation of the frequencies of banding morphs after the exclusion of unbanded shells is a strong indication that the data are genuine.



## 10. LINKAGE DISEQUILIBRIA AND THE MAINTENANCE OF POLYMORPHISM

Almost all populations of *C. nemoralis* are polymorphic for shell colour and banding. While it is broadly accepted that visual and climatic selection can cause directional changes in the genetic composition of populations, the origin and maintenance of variability itself remain unclear. Several polymorphism-protecting mechanisms have been suggested, of which the most important are: 1. heterozygote advantage, 2. frequency-dependent selection by visual predators, 3. balance of mutation and random drift, 4. selection variable over space and time coupled with migration. None of them gained universal acceptance (COOK 2005).

In *C. nemoralis*, shell colour is determined by five linked and several independent loci. In the linked group, the most important are the ground colour of the shell and the presence or absence of banding: the recombination rate is only 0.2%, with an upper 95% confidence limit of 1% (COOK & KING 1966, COOK 2005). Linkage disequilibria are common and prevail in many populations. To date, only few studies have explicitly dealt with the distribution of linkage disequilibria (GUERRUCCI-HENRION 1966, WAGNER 1990, COOK 2005), while the linkage of specific loci might be the key to the maintenance of variability; analysis of the degree of phenotype linkage disequilibrium over a wide range of conditions can shed light on polymorphism. In this chapter the direction and strength of linkage disequilibria are analysed.

### METHODS

The disequilibrium between colour and the presence or absence of bands was calculated according to the formula:

$$D = ad - bc$$

where the frequencies of the phenotypes pink unbanded (PU), yellow unbanded (YU), pink banded (PB), and yellow banded (YB) are represented by a, b, c, and d, respectively. The range of D is  $\pm 0.25$ , and equals 0 when the four combinations are present proportionally. Brown shell colour is believed to interact epistatically with the banding sometimes suppressing band expression (JONES et al. 1977); brown shells were not included in these calculations.

### RESULTS

Figure 22 presents the direction and strength of linkage disequilibria between banding and colour (pink versus yellow) along the transect, and Table 9 gives median values at open and shaded sites within each plot. Linkage disequilibria behaved in a rather erratic way. There were no significant associations between the direction and magnitude of linkage disequilibria and any climatic variable. Similarly, neither the strength nor direction of linkage disequilibria related to habitat. In general, disequilibria favoured the pink unbanded and yellow banded chro-

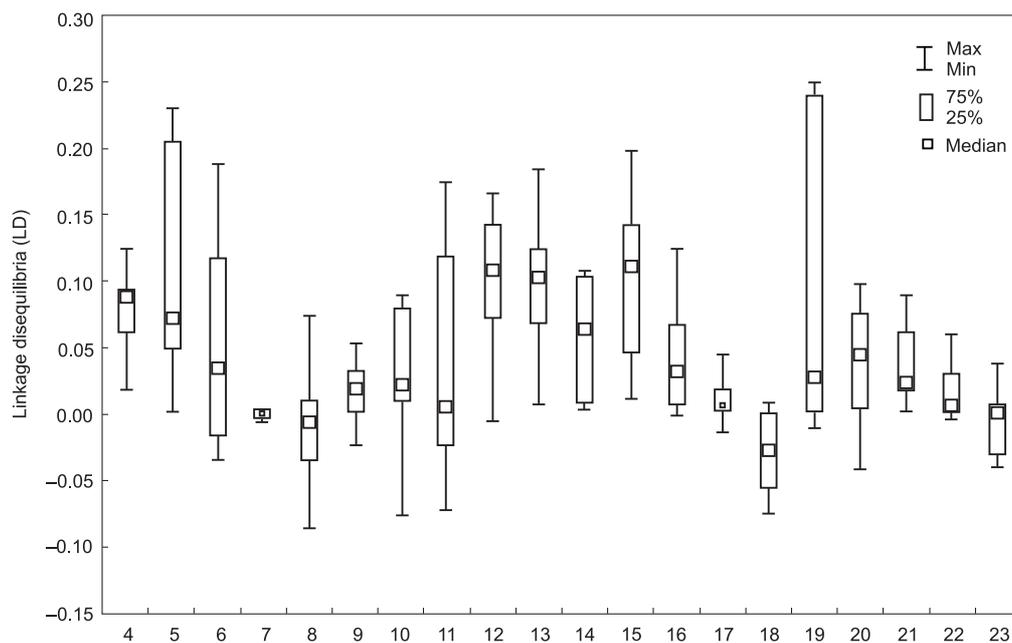


Fig. 22. Variation in the values of linkage disequilibria between colour and the presence or absence of bands along the transect (max and min without outliers). 4–23 – plot numbers

Table 9. Median values of linkage disequilibria between colour and the presence or absence of bands at open and shaded sites

Plot	Open	Shaded
4	0.092	0.085
5	0.072	0.168
6	0.002	0.045
7	0.000	0.000
8	0.000	-0.034
9	0.008	0.025
10	0.009	0.051
11	0.000	0.017
12	0.084	0.131
13	0.121	0.084
14	0.070	0.057
15	0.044	0.126
16	0.063	0.019
17	0.000	0.023
18	-0.024	-0.043
19	0.024	0.108
20	0.052	0.039
21	0.019	0.025
22	0.004	0.006
23	0.000	0.000

mosomes; 70% of samples showed an excess of unbanded in pink and 19% in yellow, while 11% were lacking one colour or banding condition altogether. In nine sites the disequilibrium was complete: all pink shells were unbanded, and all yellow shells were banded. Five of these were in plot 19. Brown shells showed a massive excess of unbanded shells relative to the other colours; of the total of 775 brown shells found in the study, only 17 (2.2%) were banded. Twelve of these came from a single sample (plot 5, sample 15) in which there were no unbanded brown shells.

## DISCUSSION

Although there were regularities in the distribution of linkage disequilibria along the transect, they were not associated with any large-scale climatic variables and showed no relation to habitat. This result is not entirely unexpected. The currently observed disequilibria represent first and foremost those present in the founding populations: when the linkage between genes is tight, and the generation time relatively long (2–3 years in *C. nemoralis* (WILLIAMSON

1979)), centuries may pass before an equilibrium is reached (COOK 2005). In the studied area the majority of populations inhabit unstable, human-impacted habitats, and often originate from recent introductions. The lack of order in the direction and strength of phenotypic disequilibria is therefore to be expected.

In *C. nemoralis*, large differences in colour and banding frequencies often exist among populations inhabiting similar habitats, even when they are geographically close. One of the reasons is that in a highly polymorphic species several different combinations of genes may be selected for under similar selective pressures. In *Cepaea*, this was theoretically predicted by GOODHART (1956), and exemplified by OŹGO & KINNISON (2008) and OŹGO (2011). The existence of a close genetic linkage between the shell colour and the presence and absence of bands restricts the possible outcomes of natural selection. While the decay of an initial disequilibrium may last for centuries, the evolutionary response of populations can be very rapid, and occur on contemporary time-scales (CAMERON & POKRYSZKO 2008, OŹGO & KINNISON 2008, OŹGO 2011): adaptation occurs in spite of genetic linkages. This means that while not all combinations of genes are equally well adapted, some variants are selected for because they are the ones that are available. For example, yellow unbanded may be the best combination in hot environments, but yellow mid-banded or three banded will have sufficiently good thermal properties to be selected for in populations where yellow is linked predominantly with banded. Similarly, pink five-banded is darker than pink unbanded, and therefore better adapted to cool environments, but pink unbanded will be selected for in populations in which pink is predominantly linked with unbanded.

Founding events play an important role in shaping morph frequencies in *Cepaea* populations. Genetic composition of the founders combined with tight linkage between loci restricts the scope of available genotypes and results in different gene combinations being selected for under similar selective regimes. COOK (1998) has shown that selection varying in space and time combined with migration can maintain polymorphism without any centripetal force. Linkage between genes reinforces this effect: not only the starting genetic pools differ among populations depending on the composition of the founders, but also the scope of available genotypes is restricted by linkages among genes. Together, these phenomena contribute to the maintenance of the striking colour and banding variation in populations of *C. nemoralis*.



## 11. SUMMARY AND CONCLUSIONS

The present paper deals with the problem of adaptation of populations of *Cepaea nemoralis* to environmental conditions. Variation in morph frequencies was analysed in a west-east transect spanning the whole range of the species in continental Europe. The line of the transect was designated along the 52°N, the longest lowland parallel in Europe. The study area spanned a distance of 1,215 km, from the North Sea coast in The Netherlands to the Bug River in Poland. Samples were collected at the crossings of the parallel with consecutive meridians, within the radius of 15 km from the crossing point. The material comprises 27,666 individuals from 235 populations. The main themes of this study are: the occurrence of *Cepaea* snails along the transect, and – in *C. nemoralis* – the pattern of distribution of morph frequencies along the transect in relation to large-scale climatic parameters and to habitat type, the effect of visual selection on morph frequencies, the amount of inter-population variation in relation to the position on the transect, variation in the level of polymorphism, the distribution of phenotypes encoded by linked and unlinked loci, and the maintenance of polymorphism in relation to linkage disequilibria. Below, the main findings of this study are summarised.

1. *C. nemoralis* occurred, with varying abundance, along the whole transect. *C. hortensis* was rarer and its distribution was more insular. Where both species were present, the areas dominated by one or the other were often clearly discernible, without any apparent differences in the habitat, which supports the hypothesis of their mutual exclusion. *C. vindobonensis* was present only in the most easterly plots and occupied habitats characteristic of *C. nemoralis* and *C. hortensis* in other areas. *C. nemoralis* was rare and not abundant in the western part of the transect, while it was common and abundant in its eastern part. The transect passes through regions used mostly for agriculture, which in western Europe is very intensive and poses a heavy impact on the environment. *C. nemoralis* is a typical eurytopic species, undemanding and easily colonising available habitats. The absence of this species from large areas within its natural range is indicative of an exceptionally severe damage to the environment.

2. Populations of *C. nemoralis* showed an immense range of variation. For all morphs, differences among plots were highly significant despite considerable variation among samples within each. Variation along the transect did not show any clear overall trends; there were some regularities, but for the most part they did not coincide with any large-scale climatic parameters. There were two significant and similar trends: pink and mid-banded tended to increase eastwards and correlated positively with the measures of continent-

ality. The trend in mid-banded is explicable on the grounds of climatic selection: there are indications that the mid-banded condition, due to some pleiotropic effect, renders the snails more resistant to climatic extremes. The trend in pink is however directly contrary to expectations under the climatic selection hypothesis, and contrasts with the patterns of spatial distribution and temporal changes in other parts of Europe. As no biologically meaningful explanation for this result is evident, and the distributions of other morphs (apart from the mid-banded) are not associated with any climatic variables, it is very likely that the distribution of morph frequencies along the transect results mostly from chance events related to recent colonisation.

3. Contrary to the large-geographic-scale, on the scale of habitats, the distribution of morph frequencies was clearly consistent with the hypothesis of climatic selection. Along the whole transect, frequencies of light shells, i.e. yellow and yellow effectively unbanded, were higher in open than in shaded habitats; shells with fused bands were more frequent in shaded habitats. Thrush anvils were rare and there were almost no differences in the frequencies of morphs between live and predated snails; the direction of differences that were significant was confounded between open and shaded sites. Thus, thrush feeding was opportunistic rather than selective, and visual selection most probably did not play an important role in shaping morph frequencies. Together, these findings indicate that the habitat-effect resulted from climatic rather than visual selection. The area encompassed by this study is under considerable, and in places strong, human pressure. Habitats are unstable, and most populations relatively short-lived. The clear habitat-effect observed in this study indicates the capability of the populations of *C. nemoralis* to adapt rapidly to changing environmental conditions. The difficulty to interpret associations of morph frequencies with large-scale climatic conditions, and contrastingly clear, repeatable and consistent with expectations response to habitat shadiness indicates that it is the details of local conditions that are crucial in shaping morph frequencies in populations of *C. nemoralis*.

4. Fusion of bands is one of the most enigmatic elements of the polymorphism of *C. nemoralis*. It is difficult to score consistently and has been rarely considered in earlier studies. In the present study fusion of bands did not show association with any large-scale climatic variables, but clearly responded to habitat shadiness: the frequencies of shells with fused bands were significantly higher in shaded than in open habitats. Interestingly, however, this response occurred only in yellow shells, in pink shells there were no dif-

ferences between habitat types. That means that the effects of climatic selection on band fusion can be expected to be statistically detectable only in populations with high frequencies of yellow five-banded shells, and explains why variation in the fusion of bands often appears to behave erratically.

5. On theoretical grounds it can be expected that the level of polymorphism will decrease towards the limits of the geographic range of the species, and that it will be lower in exposed habitats where stronger directional selection probably occurs. None of these hypotheses was borne out: there was no decrease in the level of polymorphism towards the limits of the species range, and the differences between open and shaded habitats were not consistent in direction. The first finding is explicable in view of the biology of the species: while the leptokurtic mode of dispersal might reduce the range of genetic variation, the specific mating system protects populations of this species from the impoverishment of the gene pool. Importantly, the current limits of the distribution of the species in continental Europe are not really marginal. In the west, the limit of the species range is defined by the limit of terrestrial habitats, so it is not a range limit in the ecological sense, and the species inhabits the British Isles further to the west. In the east it is dispersal capabilities rather than the extreme environmental conditions that limit the rate of the currently observed expansion of the species. The lack of consistency in the direction of differences between open and shaded habitats may indicate that selection is not really stronger in open than in shaded habitats, and does not work towards monomorphism in this habitat type. However, in the middle part of the transect, over a distance of approximately 700 km, the level of polymorphism was lower in open than in shaded habitats; this issue requires further studies.

6. In *C. nemoralis*, the loci determining the number of bands on a banded shell are independent of colour

locus and of each other. This was reflected in a strong correlation of banding morphs between yellow and pink banded shells, showing that reshuffling of alleles brings genes coded by unlinked loci to equilibrium even in relatively short-lived populations. On the other hand, the loci for the shell colour and for the presence or absence of banding are tightly linked, and this has resulted in a common occurrence of linkage disequilibria. Tight linkage between the colour and banding loci limits the scope of genotypes available to selection. This is probably one of the reasons why different populations adapt along different genetic routes to similar selective pressures and is an additional factor contributing to the maintenance of polymorphism in this strikingly variable species.

7. The present study contributes to the knowledge about the ways in which various evolutionary forces affect the genetic composition of natural populations. It shows that in contemporary continental Europe the genetic composition of populations of *C. nemoralis* results mostly from the combination of chance events related to colonization processes, restrictions posed by linkages among genes, and very localized natural selection. The central role played by chance events implies that although it is possible to explain some of the observed patterns, the predictive power of our theories is bound to remain poor.

#### ACKNOWLEDGEMENTS

My greatest thanks go to ZDZISŁAW BOGUCKI for his help and support throughout this project. I thank ROBERT A. D. CAMERON for helpful comments on the manuscript and for analysing the amount of variation within and among plots. I also thank MIRJAM HOUTLOSSER for her hospitality and help in the fieldwork in The Netherlands.

#### REFERENCES

- ARNOLD R. W. 1968. Studies on *Cepaea*. VII. Climatic selection in *Cepaea nemoralis* in the Pyrenees. Phil. Trans. Roy. Soc. London B 253: 549–593. doi: 10.1098/rstb.1968.0011
- BANTOCK C. R., PRICE D. J. 1975. Marginal populations of *Cepaea nemoralis* (L.) on the Brandon Hills, England. I. Ecology and ecogenetics. Evolution 29: 267–277. doi: 10.2307/2407215
- BELL G. 2007. Selection. The mechanism of evolution. Oxford University Press, New York.
- BROMHAM L. 2008. Reading the story in DNA. Oxford University Press, New York.
- BROOKS R. 2002. Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. Genetica 116: 343–358. doi: 10.1023/A:1021228308636
- BURLA H., GOSTELI M. 1993. Thermal advantage of pale colored morphs of the snail *Arianta arbustorum* (Helicidae, Pulmonata) in Alpine habitats. Ecography 16: 345–350. doi: 10.1111/j.1600-0587.1993.tb00223.x
- CAESAR S., AHNESJÖ J., FORSMAN A. 2007. Testing the role of coadapted genes versus bet hedging for mating strategies in colour polymorphic pygmy grasshoppers. Biol. J. Linn. Soc. 90: 491–499. doi: 10.1111/j.1095-8312.2007.00739.x
- CAIN A. J. 1977. The uniqueness of the polymorphism of *Cepaea* (Pulmonata: Helicidae) in western Europe. J. Conch. 29: 129–136.



- CAIN A. J. 1983. Ecology and ecogenetics of terrestrial molluscan populations. In: RUSSELL-HUNTER W. D. (ed.) *The Mollusca*. Vol. 6. Ecology, Academic Press, New York, pp. 597–647.
- CAIN A. J., CURREY J. D. 1963. Differences in interactions between selective forces acting in the wild on certain pleiotropic genes of *Cepaea*. *Nature* 197: 411–412. doi: 10.1038/197411a0
- CAIN A. J., KING J. M. B., SHEPPARD P. M. 1960. New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L. *Genetics* 45: 393–411.
- CAIN A. J., SHEPPARD P. M. 1950. Selection in the polymorphic land snail *Cepaea nemoralis*. *Heredity* 4: 275–294. doi: 10.1038/hdy.1950.22
- CAIN A. J., SHEPPARD P. M. 1954. Natural selection in *Cepaea*. *Genetics* 39: 89–116.
- CAMERON R. A. D. 1997. *Cepaea* research 1900–1950: too many problems for a solution? *Arch. Nat. Hist.* 25: 401–412. doi: 10.3366/anh.1998.25.3.401
- CAMERON R. A. D. 2001. *Cepaea nemoralis* in a hostile environment: continuity, colonizations and morph-frequencies over time. *Biol. J. Linn. Soc.* 74: 255–264. doi: 10.1111/j.1095-8312.2001.tb01390.x
- CAMERON R. A. D., COOK L. M. 2012. Habitat and the shell polymorphism of *Cepaea nemoralis* (L.): interrogating the Evolution Megalab database. *J. Moll. Stud.* doi: 10.1093/mollus/eyr052
- CAMERON R. A. D., OZGO M., HORSÁK M., BOGUCKI Z. 2011. At the north-eastern extremity: variation in *Cepaea nemoralis* around Gdańsk, northern Poland. *Biologia* 66: 1097–1113. doi: 10.2478/s11756-011-0128-9
- CAMERON R. A. D., PANNETT D. J. 1985. Interaction between area effects and variation with habitat in *Cepaea*. *Biol. J. Linn. Soc.* 24: 365–379. doi: 10.1111/j.1095-8312.1985.tb00382.x
- CAMERON R. A. D., POKRYSZKO B. M. 2008. Variation in *Cepaea* populations over 42 years: climate fluctuations destroy a topographical relationship of morph-frequencies. *Biol. J. Linn. Soc.* 95: 53–61. doi: 10.1111/j.1095-8312.2008.01042.x
- CAMERON R. A. D., POKRYSZKO B. M., HORSÁK M. 2009. Contrasting patterns of variation in urban populations of *Cepaea* (Gastropoda: Pulmonata): a tale of two cities. *Biol. J. Linn. Soc.* 97: 27–39. doi: 10.1111/j.1095-8312.2008.01187.x
- CHANG H.-W. 1991. Activity and weight loss in relation to solar radiation in the polymorphic land snail *Cepaea nemoralis*. *J. Zool. (Lond.)* 255: 213–225. doi: 10.1111/j.1469-7998.1991.tb03812.x
- CLARKE B. C. 1960. Divergent effects of natural selection on two closely-related polymorphic snails. *Heredity* 14: 423–443. doi: 10.1038/hdy.1960.39
- CLARKE B. C., ARTHUR W., HORSLEY D. T., PARKIN D. T. 1978. Genetic variation and natural selection in pulmonate snails. In: FRETTER V., PEAKE J. (eds). *Pulmonates*. Academic Press, New York, pp. 220–270.
- CLARKE B. C., MURRAY J. J. 1962a. Changes in gene frequency in *Cepaea nemoralis* (L.). *Heredity* 17: 445–465. doi: 10.1038/hdy.1962.47
- CLARKE B. C., MURRAY J. J. 1962b. Changes in gene frequency in *Cepaea nemoralis* (L.); the estimation of selective values. *Heredity* 17: 467–476. doi: 10.1038/hdy.1962.48
- COOK L. M. 1998. A two-stage model for *Cepaea* polymorphism. *Phil. Trans. R. Soc. Lond. B* 353: 1577–1593. doi: 10.1098/rstb.1998.0311
- COOK L. M. 2005. Disequilibrium in some *Cepaea* populations. *Heredity* 94: 497–500. doi: 10.1038/sj.hdy.6800645
- COOK L. M. 2008. Variation with habitat in *Cepaea nemoralis*: the Cain & Sheppard diagram. *J. Moll. Stud.* 74: 239–243. doi: 10.1093/mollus/eyn011
- COOK L. M., KING J. M. B. 1966. Some data on the genetics of shell-character polymorphism in the snail *Arianta arbustorum*. *Genetics* 53: 415–425.
- COOK L. M., PETTIT C. W. A. 1998. Morph frequencies in the snail *Cepaea nemoralis*: changes with time and their interpretation. *Biol. J. Linn. Soc.* 64: 137–150.
- DVOŘÁK L., HONEK A. 2004. The spreading of the Brown Lipped Snail, *Cepaea nemoralis*, in the Czech Republic. *J. Nat. Mus., Nat. Hist. Ser.* 173: 97–103.
- ELLERS J., BOGGS C. L. 2002. The evolution of wing color in *Colias* butterflies: heritability, sex linkage, and population divergence. *Evolution* 56: 836–840.
- ELLERS J., BOGGS C. L. 2004. Functional ecological implications of intraspecific differences in wing melanization in *Colias* butterflies. *Biol. J. Linn. Soc.* 82: 79–87. doi: 10.1111/j.1095-8312.2004.00319.x
- ENDLER J. A. 1986. *Natural selection in the wild*. Princeton University Press, Princeton, New Jersey. doi: 10.1046/j.1420-9101.1995.8010053.x
- FORSMAN A. 1995. Opposing fitness consequences of colour pattern in male and female snakes. *J. Evol. Biol.* 8: 53–70
- FUTUYMA D. 2005. *Evolutionary Biology*. Sinauer Associates, Inc. Sunderland, Massachusetts.
- GAMBLE S., LINDHOLM A. K., ENDLER J. A., BROOKS R. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecol. Lett.* 6: 463–472. doi: 10.1046/j.1461-0248.2003.00449.x
- GASTON K. J., FULLER R. A. 2007. Biodiversity and extinction: losing the common and the widespread. *Prog. Phys. Geogr.* 31: 213–225. doi: 10.1177/0309133307076488
- GASTON K. J., FULLER R. A. 2008. Commonness, population depletion and conservation biology. *Trends Ecol. Evol.* 23: 14–19. doi: 10.1016/j.tree.2007.11.001
- GOODHART C. B. 1956. Genetic stability in populations of the polymorphic snail, *Cepaea nemoralis* (L.). *Proc. Linn. Soc. London* 167: 50–67. doi: 10.1111/j.1095-8312.1956.tb00766.x
- GOODHART C. B. 1958. Genetic stability in populations of the polymorphic snail, *Cepaea nemoralis* (L.): a further example. *Proc. Linn. Soc. London* 169: 163–167. doi: 10.1111/j.1095-8312.1958.tb00820.x
- GOODHART C. B. 1987. Why are some snails visibly polymorphic and others not? *Biol. J. Linn. Soc.* 31: 35–58. doi: 10.1111/j.1095-8312.1987.tb01979.x
- GOSDEN T. P., STOKS R., SVENSSON E. I. 2011. Range limits, large-scale biogeographic variation, and localized evolu-

- tionary dynamics in a polymorphic damselfly. *Biol. J. Linn. Soc.* 102: 775–785. doi: 10.1111/j.1095-8312.2011.01619.x
- GUERRUCCI-HENRION M. A. 1966. Recherches sur les populations naturelles de *Cepaea nemoralis* en Bretagne. *Arch. Zool. Exp. Gen.* 107: 369–417.
- HARLEY C. D. G., PANKEY M. S., WARES J. P., GROSBURG R. K., WONHAM M. J. 2006. Color polymorphism and genetic structure in the sea star *Pisaster ochraceus*. *Biol. Bull.* 211: 248–262. doi: 10.2307/4134547
- HEATH D. J. 1975. Colour, sunlight and internal temperatures in the land snail *Cepaea nemoralis* (L.). *Oecologia* (Berl.) 19: 29–38. doi: 10.1007/BF00377587
- HOEKSTRA H. E., KRENZ J. G., NACHMAN M. W. 2005. Local adaptation in the rock pocket mouse (*Chaetodipus intermedius*): natural selection and phylogenetic history of populations. *Heredity* 94: 217–228. doi: 10.1038/sj.hdy.6800600
- HONEK A. 1995. Geographic distribution and shell colour and banding polymorphism in marginal populations of *Cepaea nemoralis* (Gastropoda, Helicidae). *Malacologia* 37: 111–122.
- IBRAHIM K. M., NICHOLS R. A., HEWITT G. M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* 77: 282–291. doi: 10.1038/hdy.1996.142
- JONES J. S. 1973. Ecological genetics and natural selection in molluscs. *Science* 1182: 546–552. doi: 10.1126/science.182.4112.546
- JONES J. S., LEITH B. H., RAWLINGS P. 1977. Polymorphism in *Cepaea*: a problem with too many solutions? *Ann. Rev. Ecol. Syst.* 8: 109–143. doi: 10.1146/annurev.es.08.110177.000545
- KARK S., WARBURG I., WERNER Y. L. 1997. Polymorphism in the snake *Psammophis schokari* on both sides of the desert edge in Israel and Sinai. *J. Arid Env.* 37: 513–527. doi: 10.1006/jare.1997.0296
- KERNEY M. P., CAMERON R. A. D. 2006. Guide des escargots et limaces d'Europe. Delachaux et Niestlé, Paris.
- KRZANOWSKA H., ŁOMNICKI A., RAFIŃSKI J. 1982. Wprowadzenie do genetyki populacji. PWN, Warszawa.
- KRZANOWSKA H., ŁOMNICKI A., RAFIŃSKI J., SZARSKI H., SZYMURA J. M. 2002. Zarys mechanizmów ewolucji. PWN, Warszawa.
- LAMOTTE M. 1954. Sur le déterminisme génétique du polymorphisme chez *Cepaea nemoralis* L. *C. R. Acad. Sci.* 239: 365–367.
- LAMOTTE M. 1959. Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spring Harbor Symp. Quant. Biol.* 34: 65–86. doi: 10.1101/SQB.1959.024.01.009
- LINDSTEDT C., LINDSTRÖM L., MAPPE J. 2009. Thermoregulation constrains effective warning signal expression. *Evolution* 63: 469–478. doi: 10.1111/j.1558-5646.2008.00561.x
- MCKILLUP S. C., MCKILLUP R. V. 2008. Apostasy and selection for crypsis in the marine snail *Littoraria filosa*: an explanation for a balanced colour polymorphism. *Biol. J. Linn. Soc.* 95: 62–71. doi: 10.1111/j.1095-8312.2008.01032.x
- MILLSTEIN R. L. 2008. Distinguishing drift and selection empirically: “The Great Snail Debate” of the 1950s. *J. Hist. Biol.* 41: 339–367. doi: 10.1007/s10739-007-9145-5
- MILLSTEIN R. L. 2009. Concepts of drift and selection in “The Great Snail Debate” of the 1950s and Early 1960s. *Trans. Am. Phil. Soc.* 99: 271–298.
- MULLEN L. M., VIGNIERI S. N., GORE J. A., HOEKSTRA H. E. 2009. Adaptive basis of geographic variation: genetic, phenotypic and environmental differences among beach mouse populations. *Proc. R. Soc. Lond. B* 276: 3809–3818. doi: 10.1098/rspb.2009.1146
- MURRAY J. J. 1964. Multiple mating and effective population size in *Cepaea nemoralis*. *Evolution* 18: 283–291. doi: 10.2307/2406402
- MURRAY J. J. 1975. The genetics of mollusca. In: KING R. C. (ed.). *Handbook of genetics*. Vol. 3, Plenum, New York, pp. 3–31.
- NOSIL P. 2009. Adaptive population divergence in cryptic color-pattern following a reduction in gene flow. *Evolution* 63: 1902–1912. doi: 10.1111/j.1558-5646.2009.00671.x
- OŻGO M. 2005a. Polimorfizm wstężyka gajowego (*Cepaea nemoralis* L.): od selekcji wizualnej po zmienność molekularną. *Przeegl. Zool.* 49: 7–30.
- OŻGO M. 2005b. *Cepaea nemoralis* (L.) in southeastern Poland: association of morph frequencies with habitat. *J. Moll. Stud.* 71: 93–103. doi: 10.1093/mollus/eyi012
- OŻGO M. 2008. Current problems in the research of *Cepaea* polymorphism. *Folia Malacol.* 16: 55–60.
- OŻGO M. 2011. Rapid evolution in unstable habitats: a success story of the polymorphic land snail *Cepaea nemoralis* (Gastropoda: Pulmonata). *Biol. J. Linn. Soc.* 102: 251–262. doi: 10.1111/j.1095-8312.2010.01585.x
- OŻGO M., BOGUCKI Z. 2006. Shell predation and cannibalism in land snails living on acid and calcium-deficient soils. *Folia Malacol.* 14: 217–220.
- OŻGO M., BOGUCKI Z. 2011. Colonization, stability, and adaptation in a transplant experiment of the polymorphic land snail *Cepaea nemoralis* (Gastropoda: Pulmonata) at the edge of its geographical range. *Biol. J. Linn. Soc.* 104: 462–470. doi: 10.1111/j.1095-8312.2011.01732.x
- OŻGO M., KINNISON M. T. 2008. Contingency and determinism during convergent contemporary evolution in the polymorphic land snail, *Cepaea nemoralis*. *Evol. Ecol. Res.* 10: 721–733.
- OŻGO M., KOMOROWSKA A. 2009. Shell banding polymorphism in *Cepaea vindobonensis* in relation to habitat in south-eastern Poland. *Malacologia* 51: 81–88. doi: 10.4002/040.051.0105
- OŻGO M., SCHILTHUIZEN M. 2011. Evolutionary change in *Cepaea nemoralis* shell colour over 43 years. *Global Change Biol.* doi: 10.1111/j.1365-2486.2011.02514.x
- RAMOS M. A. 1985. Shell polymorphism in a southern peripheral population of *Cepaea nemoralis* (L.) (Pulmonata: Helicidae) in Spain. *Biol. J. Linn. Soc.* 25: 197–208. doi: 10.1111/j.1095-8312.1985.tb00392.x
- RICHARDS A. V., MURRAY J. J. 1975. The relation of phenotype to habitat in an introduced colony of *Cepaea nemoralis*. *Heredity* 34: 128–131. doi: 10.1038/hdy.1975.13



- RICHARDSON A. M. M. 1974. Differential climatic selection in natural populations of the land snail *Cepaea nemoralis*. *Nature* 247: 572–573. doi: 10.1038/247572a0
- RIEDEL A. 1988. Ślimaki łądowe. Gastropoda terrestria. Katalog Fauny Polski 36. PWN, Warszawa.
- ROTARIDES M. 1926. Über die Bandvariation von *Cepaea vindobonensis* Fer. *Zool. Anz.* 67: 28–44.
- SCHILTHUIZEN M. 2001. Frogs, flies and dandelions. Speciation – The Evolution of New Species. Oxford University Press, New York.
- SHEPPARD P. M. 1951. Fluctuations in the selective value of certain phenotypes in the polymorphic land snail *Cepaea nemoralis* (L.). *Heredity* 5: 125–134. doi: 10.1038/hdy.1951.8
- SILVERTOWN J., COOK L., CAMERON R., DODD M., MCCONWAY K., WORTHINGTON J., SKELTON P., ANTON C., BOSSDORF O., BAUR B., SCHILTHUIZEN M., FONTAINE B., SATTMANN H., BERTORELLE G., CORREIA M., OLIVEIRA C., POKRYSZKO B., OŽGO M., STALAŽS A., GILL E., RAMMUL Ü., SÓLYMOS P., FÉHER Z., JUAN X. 2011. Citizen science reveals unexpected continental-scale evolutionary change in a model organism. *PLoS ONE* 6: e18927. doi: 10.1371/journal.pone.0018927
- SIMPSON E. H. 1949. The measurement of diversity. *Nature* 163: 688. doi: 10.1038/163688a0
- SOULÉ M. 1973. The epistasis cycle: a theory of marginal populations. *Annu. Rev. Ecol. Syst.* 4: 165–187. doi: 10.1146/annurev.es.04.110173.001121
- SULIKOWSKA-DROZD A. 2008. Ślimakowate (Helicidae). In: BOGDANOWICZ W., CHUDZICKA E., PILIPUK I., SKIBIŃSKA E. (eds). *Fauna of Poland – characteristics and checklists of species*. Vol. 3, Muzeum i Instytut Zoologii PAN, Warszawa, pp. 399–400.
- SVERLOVA N. 2002. Einschleppung und Polymorphismus der *Cepaea*-Arten am Beispiel von Lwow in der Westukraine (Gastropoda: Pulmonata: Helicidae). *Malak. Abh.* 20: 267–274.
- TANAKA K. 2007. Thermal biology of a colour-dimorphic snake, *Elaphe quadrivirgata*, in a montane forest: do melanistic snakes enjoy thermal advantages? *Biol. J. Linn. Soc.* 92: 309–322. doi: 10.1111/j.1095-8312.2007.00849.x
- TILLING S. M. 1985a. The effect of interspecific interaction on spatial distribution patterns in experimental populations of *Cepaea nemoralis* (L.) and *C. hortensis* (Muell.). *Biol. J. Linn. Soc.* 24: 71–81. doi: 10.1111/j.1095-8312.1985.tb00161.x
- TILLING S. M. 1985b. The effects of density and interspecific interaction of mortality in experimental populations of adult *Cepaea* (Held.). *Biol. J. Linn. Soc.* 24: 61–70. doi: 10.1111/j.1095-8312.1985.tb00160.x
- TRULLAS S. C., VAN WYK J. H., SPOTILA J. R. 2007. Thermal melanism in ectotherms. *J. Therm. Biol.* 32: 235–245. doi: 10.1016/j.jtherbio.2007.01.013
- UNSICKER S. B., KÖHLER G., LINZ J., STEIN C., WEISSER W. W. 2008. Colour morph related performance in the meadow grasshopper *Chorthippus parallelus* (Orthoptera, Acrididae). *Ecol. Entomol.* 33: 631–637. doi: 10.1111/j.1365-2311.2008.01015.x
- VENCES M., GALAN P., VIEITES D. R., PUENTE M., OETTER K., WANKE S. 2002. Field body temperatures and heating rates in a montane frog population: the importance of black dorsal pattern for thermoregulation. *Ann. Zool. Fenn.* 39: 209–220.
- WAGNER A. 1990. Linkage disequilibrium in *Cepaea nemoralis* (L.) in Poland. *Genetica Polonica* 31: 223–228.
- WANG I. J., SHAFFER H. B. 2008. Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. *Evolution* 62: 2742–2759. doi: 10.1111/j.1558-5646.2008.00507.x
- WILLIAMSON P. 1979. Age determination of juvenile and adult *Cepaea*. *J. Moll. Stud.* 45: 52–60.
- WOLDA H. 1963. Natural populations of the polymorphic land snail *Cepaea nemoralis* (L.). *Arch. Neerl. Zool.* 15: 381–471. doi: 10.1163/036551663X00014
- WOLDA H. 1965. Some preliminary observations on the distribution of the various morphs within natural populations of the polymorphic landsnail *Cepaea nemoralis* (L.). *Arch. Neerl. Zool.* 16: 280–292. doi: 10.1163/036551665X00021
- WOLDA H. 1969. Genetics of polymorphism in the land snail, *Cepaea nemoralis*. *Genetica* 40: 475–502. doi: 10.1007/BF01787373
- WOOLBRIGHT L. L., STEWART M. M. 2008. Spatial and temporal variation in color pattern morphology in the tropical frog *Eleutherodactylus coqui*. *Copeia* 2: 431–437. doi: 10.1643/CG-06-092
- WRIGHT S. 1978. *Evolution and the genetics of populations* vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago, IL.

Received: December 15th, 2011

Revised: February 20th, 2012

Accepted: March 23rd, 2012



Appendix 1. Details of sampling sites and sample composition. U – unbanded, M – mid-banded, T – three-banded, F – five-banded, O – other banding forms, B – banded. Habitat: O – open, S – shaded

Meridian No.	Population No.	Geographic coordinates		Habitat	Yellow					Pink					Brown		Total
					U	M	T	F	O	U	M	T	F	O	U	B	
4	1	52.0269	4.1678	S	1	12	3	16	0	60	19	2	28	0	0	0	141
4	2	52.0308	4.1675	O	11	23	2	31	1	25	11	0	26	0	0	0	130
4	3	52.0256	4.1689	O	3	7	0	16	0	70	4	0	28	0	0	0	128
4	4	52.0339	4.1725	O	15	8	11	41	0	34	5	0	8	0	0	0	122
4	5	52.0333	4.1711	O	13	7	5	42	0	33	4	2	19	0	0	0	125
4	6	51.9831	4.1356	S	12	12	1	22	1	16	20	5	28	0	3	0	120
4	7	51.9775	4.1358	S	6	12	1	17	0	13	2	0	0	0	0	0	51
4	8	51.9739	4.1397	S	3	4	1	21	0	29	6	1	25	0	2	0	92
4	9	51.9761	4.1383	O	5	2	1	12	0	6	2	0	3	0	0	0	31
4	10	52.0353	4.2117	S	8	13	5	7	1	14	11	9	11	0	0	0	79
4	11	52.0283	4.1683	S	7	12	1	13	0	16	7	0	5	0	0	0	61
5	1	52.1022	4.9194	S	2	16	24	17	2	10	5	7	6	1	0	0	90
5	2	52.1033	4.9203	S	1	20	3	27	0	45	3	1	0	0	4	0	104
5	3	52.0275	4.9047	O	1	10	124	12	2	10	0	14	2	0	11	0	186
5	4	51.9844	4.9114	S	19	22	0	53	0	63	0	0	0	0	0	0	157
5	5	52.0367	5.0372	O	0	6	31	16	1	5	1	2	4	0	3	0	69
5	6	52.0375	5.0378	S	1	33	12	11	1	38	3	5	9	0	6	0	119
5	7	52.0369	5.0375	O	1	33	9	10	0	57	6	1	3	0	3	0	123
5	8	52.0172	4.9547	O	3	59	2	37	0	19	1	0	1	0	2	0	124
5	9	51.9322	4.8906	O	0	4	15	6	0	14	0	0	0	0	33	1	73
5	10	51.9317	4.9325	S	25	15	35	19	1	8	2	13	6	0	4	0	128
5	11	51.9389	4.9739	S	0	35	0	12	0	83	0	0	2	0	0	0	132
5	12	52.0575	4.9058	O	2	18	12	19	0	5	8	6	6	1	7	0	84
5	13	52.0444	4.8947	O	1	37	29	18	3	11	4	4	6	1	5	0	119
5	14	51.9844	4.9092	O	42	29	10	22	0	17	0	0	0	0	6	0	126
5	15	52.0311	4.8650	S	0	26	14	20	0	11	23	12	14	1	0	12	133
6	1	52.0556	6.1719	S	72	0	10	20	3	2	0	0	0	0	16	0	123
6	2	52.0597	6.1767	S	19	0	38	14	13	4	0	0	0	0	47	0	135
6	3	52.0336	6.1753	O	50	1	20	15	1	36	0	1	1	0	0	0	125
6	4	52.0286	6.1847	O	33	0	34	14	0	39	0	2	1	0	0	0	123
6	5	52.0244	6.1592	O	8	0	48	18	3	1	0	26	8	1	28	0	141
6	6	52.0125	6.1653	S	14	5	7	9	0	88	0	0	0	0	0	0	123
6	7	52.0469	6.1592	O	68	1	17	12	0	2	0	3	2	0	24	0	129
6	8	52.0456	6.1306	O	21	0	23	32	0	5	0	13	22	0	7	0	123
6	9	52.0428	6.1633	S	26	0	8	46	0	8	0	0	0	0	45	0	133
6	10	51.9583	6.1344	O	0	0	11	5	0	26	0	2	3	0	0	0	47
6	11	51.9733	6.1319	O	108	0	12	2	0	2	0	0	0	0	0	0	124
7	1	52.0342	7.2022	O	1	4	1	10	0	6	0	0	1	0	0	0	23
7	2	52.0750	7.1436	O	0	4	48	58	12	0	0	0	0	0	0	0	122
7	3	52.0717	7.1453	O	51	13	2	34	9	5	4	1	12	0	0	0	131
7	4	52.0728	7.1469	O	1	21	12	72	24	0	1	0	0	0	0	0	131
7	5	52.0706	7.1519	S	2	19	2	86	2	1	0	0	11	0	0	0	123



Meridian No.	Population No.	Geographic coordinates		Habitat	Yellow					Pink					Brown		Total
					U	M	T	F	O	U	M	T	F	O	U	B	
7	6	52.0761	7.1367	S	0	7	0	36	0	0	5	0	7	0	0	0	55
7	7	52.0769	7.1411	S	0	10	2	22	2	0	0	2	4	0	0	0	42
7	8	52.0778	7.1378	O	3	8	16	39	0	1	3	11	38	2	0	0	121
8	1	52.0114	7.9994	O	79	8	0	16	0	0	0	0	0	0	0	0	103
8	2	52.0269	8.0100	S	20	8	2	15	2	26	25	8	36	0	0	0	142
8	3	52.0269	8.0094	O	53	3	5	10	0	24	6	3	26	1	0	0	131
8	4	52.0142	7.9481	O	11	0	6	10	4	26	1	11	47	4	4	0	124
8	5	52.0136	7.9456	O	10	1	15	28	0	8	3	12	12	0	0	0	89
8	6	52.0708	7.8794	S	0	8	1	19	1	8	3	0	6	1	0	0	47
8	7	52.0664	8.0378	O	39	0	0	5	0	0	0	0	0	0	0	0	44
8	8	52.0058	8.0392	S	42	2	0	1	0	81	15	1	9	0	3	0	154
8	9	51.9725	7.8992	O	55	6	7	2	0	34	3	15	2	0	0	0	124
8	10	51.9656	7.8694	O	18	22	4	29	11	24	6	3	7	4	13	0	141
9	1	52.0044	8.9953	S	0	1	2	22	0	3	0	3	8	0	0	0	39
9	2	52.0050	8.9956	S	7	3	19	50	6	3	0	4	24	6	0	0	122
9	3	52.0053	8.9956	O	2	3	37	43	7	4	0	9	20	2	0	0	127
9	4	51.9944	8.9936	S	15	1	16	58	3	15	0	6	13	1	0	0	128
9	5	51.9742	8.9897	O	8	47	43	19	0	0	3	1	0	0	0	0	121
9	6	51.9814	9.0539	O	4	0	0	8	0	81	2	0	23	7	0	0	125
9	7	51.9900	9.0086	S	54	1	5	39	9	11	0	0	4	0	0	0	123
9	8	52.0078	9.0728	S	11	2	0	24	0	2	0	0	4	0	0	0	43
9	9	52.0094	9.0750	O	34	4	11	45	0	1	0	4	5	0	0	0	104
10	1	51.9683	9.8061	O	0	34	7	55	0	0	9	3	16	0	0	0	124
10	2	51.9825	9.8206	S	5	11	18	48	2	16	2	2	9	2	0	0	115
10	3	52.0653	9.8350	S	3	0	24	27	7	7	0	21	13	10	0	0	112
10	4	52.0481	9.9369	O	0	6	7	37	6	11	6	1	40	7	0	0	121
10	5	52.0408	9.9344	S	0	0	12	71	0	18	0	5	31	0	0	0	137
10	6	52.0922	10.0500	O	54	15	0	36	1	2	0	0	0	0	0	0	108
10	7	52.0178	10.0061	O	55	7	1	26	0	12	12	0	20	0	0	0	133
10	8	51.9861	10.0228	O	8	0	73	49	4	6	0	1	0	0	0	0	141
10	9	51.9761	9.9497	S	0	0	0	69	1	2	0	0	53	1	0	0	126
10	10	51.9981	9.9425	S	1	0	21	67	3	17	0	9	13	0	0	0	131
10	11	51.9956	9.9514	S	10	0	5	21	0	10	3	2	13	0	0	0	64
11	1	52.1014	10.9258	O	13	0	1	4	0	6	0	3	5	0	0	0	32
11	2	52.1011	10.9256	O	33	0	3	0	0	15	0	5	0	0	0	0	56
11	3	52.1069	10.8811	O	68	0	15	22	2	6	0	3	6	0	0	0	122
11	4	52.0958	10.8631	S	3	0	31	22	0	45	0	14	10	0	0	0	125
11	5	52.0839	10.8331	S	45	0	22	21	1	21	0	8	3	0	0	0	121
11	6	52.0683	11.0994	S	12	1	48	26	5	5	0	18	8	1	0	0	124
11	7	52.0836	11.1178	S	7	0	79	10	0	40	0	5	1	0	0	0	142
11	8	52.1050	11.0547	S	24	5	22	33	0	15	3	12	23	0	0	0	137
11	9	52.1092	11.0572	S	41	0	17	24	1	12	0	12	22	2	0	0	131
11	10	52.1094	11.0572	O	30	0	17	25	0	28	0	10	16	0	0	0	126
11	11	51.9536	11.1639	O	7	4	50	5	2	14	0	1	1	0	0	0	84

Meridian No.	Population No.	Geographic coordinates		Habitat	Yellow					Pink					Brown		Total
					U	M	T	F	O	U	M	T	F	O	U	B	
11	12	51.9081	11.0408	O	17	2	30	8	0	23	0	2	0	0	0	0	82
11	13	51.9856	10.9203	O	100	4	35	14	2	0	0	0	0	0	0	0	155
12	1	52.1169	12.0686	S	0	14	6	25	0	15	15	7	39	0	0	0	121
12	2	52.1142	12.0936	O	0	19	14	13	0	62	7	9	8	1	0	0	133
12	3	52.0789	11.8311	O	4	5	34	42	0	1	8	10	23	0	0	0	127
12	4	52.0644	11.8047	O	1	1	8	55	16	42	0	5	12	2	0	0	142
12	5	52.0392	11.7822	O	4	14	1	29	0	36	18	1	26	0	0	0	129
12	6	51.9764	12.0622	S	0	37	1	18	0	44	19	0	18	0	0	0	137
12	7	51.9714	12.0611	O	0	40	3	8	1	24	47	3	7	0	0	0	133
12	8	51.9714	12.0600	S	0	42	4	9	0	41	25	3	2	0	0	0	126
12	9	51.9608	12.0679	S	0	19	2	4	0	55	41	1	13	0	0	0	135
12	10	52.0631	11.7994	S	4	11	5	25	0	30	6	2	8	0	0	0	91
13	1	51.9858	13.1850	O	0	43	0	3	0	56	3	0	3	0	22	0	130
13	2	52.0086	13.0853	O	3	29	3	5	0	44	22	3	5	0	16	0	130
13	3	51.9994	13.0850	S	0	12	0	3	0	92	18	0	2	0	5	0	132
13	4	52.0014	13.0503	S	0	13	0	8	0	86	13	0	3	0	1	0	124
13	5	51.9750	12.9753	O	0	25	0	17	1	48	18	1	19	0	0	0	129
13	6	51.9750	12.9756	S	0	14	1	23	0	34	17	7	30	0	0	0	126
13	7	52.0911	12.9414	O	0	0	2	2	0	51	19	6	42	4	0	0	126
13	8	52.0900	13.1361	S	0	12	27	13	0	64	4	9	4	1	0	0	134
13	9	52.0900	13.1372	S	0	18	9	9	1	28	32	11	14	2	0	0	124
13	10	52.0908	12.8681	S	0	2	23	6	4	4	10	59	14	9	0	0	131
14	1	52.0158	14.1956	S	0	4	0	12	1	41	26	2	35	0	0	0	121
14	2	52.0192	14.1989	S	1	11	2	3	1	54	32	3	14	2	0	0	123
14	3	52.0267	14.1958	O	1	13	16	9	0	6	17	50	26	0	0	0	138
14	4	52.0289	14.2042	O	0	34	1	23	1	29	18	3	16	1	0	0	126
14	5	52.0286	14.2042	S	0	46	1	16	1	26	26	3	6	0	0	0	125
14	6	52.0453	14.1367	O	0	5	0	0	0	2	36	0	9	1	0	0	53
14	7	52.1061	14.1264	O	1	15	0	15	0	45	30	0	30	0	1	0	137
14	8	52.1553	14.1622	S	1	5	48	16	0	25	4	21	8	0	0	0	128
14	9	52.0539	13.9825	O	1	75	1	17	4	15	9	0	2	0	0	0	124
14	10	52.0306	13.8833	S	0	3	0	0	0	41	52	15	22	0	0	0	133
15	1	52.0392	14.8689	O	0	54	0	0	0	37	35	0	1	0	0	0	127
15	2	52.0406	14.8644	S	0	4	0	13	2	41	25	0	47	0	0	0	132
15	3	51.9844	14.7931	S	0	16	0	0	0	111	0	0	0	0	0	0	127
15	4	52.0758	14.8589	O	0	5	0	70	6	10	7	0	38	0	0	0	136
15	5	52.0714	14.8997	S	0	1	0	5	0	34	5	0	6	0	0	0	51
15	6	52.0539	14.8942	S	0	0	0	35	0	93	0	0	0	0	0	0	128
15	7	52.0828	14.8675	S	0	20	0	50	1	46	6	0	17	1	0	0	141
15	8	52.0583	14.8969	O	1	0	0	97	3	19	2	0	5	0	0	0	127
15	9	52.0678	14.9075	S	0	7	0	31	0	62	6	0	23	0	1	0	130
15	10	52.0819	14.8744	O	0	7	0	61	2	1	0	0	0	0	0	0	71
15	11	52.0417	14.8639	O	0	18	0	8	1	7	75	0	19	2	0	0	130
15	12	52.0417	14.8622	S	1	19	0	3	0	56	38	0	6	0	0	0	123



Meridian No.	Population No.	Geographic coordinates		Habitat	Yellow					Pink					Brown		Total
					U	M	T	F	O	U	M	T	F	O	U	B	
15	13	51.9875	14.8003	S	0	51	0	16	0	50	13	0	1	0	0	0	131
16	1	52.1003	16.1122	O	0	24	73	42	1	0	0	0	0	0	0	0	140
16	2	52.0842	16.1242	S	2	0	5	4	0	9	12	11	0	0	25	0	68
16	3	52.0756	16.1281	S	10	28	31	20	0	3	12	8	5	0	24	0	141
16	4	52.0758	16.1272	S	1	33	17	23	1	1	10	2	7	0	10	0	105
16	5	52.0964	15.9167	O	0	30	3	16	0	30	46	4	24	0	0	0	153
16	6	52.1242	16.0533	S	0	19	0	0	0	26	56	3	4	0	0	0	108
16	7	52.0778	15.8639	S	0	17	13	30	0	36	14	3	19	0	0	0	132
16	8	52.0742	15.8628	O	0	36	15	3	0	30	26	11	8	0	0	0	129
16	9	52.0747	15.8625	S	0	23	3	4	0	40	50	8	8	2	0	0	138
16	10	52.0736	15.8647	O	1	12	1	3	0	17	56	2	29	0	0	0	121
16	11	52.0733	15.8647	S	0	4	1	1	0	20	64	0	41	0	0	0	131
16	12	52.0603	15.8206	O	0	60	0	5	0	1	55	0	0	0	0	0	121
16	13	52.0669	15.8617	O	0	22	7	7	0	34	36	9	19	0	0	0	134
16	14	51.9594	15.8067	O	0	12	4	1	1	50	20	0	3	0	0	0	91
17	1	51.900	17.0111	O	0	42	0	58	1	2	0	0	1	0	39	0	143
17	2	51.8842	17.0111	O	0	27	0	49	5	3	18	0	24	0	3	0	129
17	3	51.8839	17.0119	S	0	79	1	6	1	26	10	0	2	0	10	0	135
17	4	51.8811	17.0006	S	0	5	0	25	1	31	1	0	4	0	65	0	132
17	5	51.8792	17.0028	S	0	16	0	45	1	11	11	0	37	2	13	0	136
17	6	51.9792	17.0097	O	0	1	0	23	0	0	0	0	47	1	0	0	72
17	7	51.9392	16.9631	S	0	17	0	18	0	0	24	0	13	0	0	0	72
17	8	52.0678	17.0536	O	19	0	0	36	1	0	0	0	0	0	0	0	56
17	9	51.9956	16.8575	O	0	16	0	22	1	0	26	0	65	2	0	0	132
17	10	52.0056	16.8528	O	1	77	4	34	8	0	0	0	0	0	0	0	124
17	11	52.0078	16.8503	O	0	25	8	19	0	0	0	0	0	0	0	0	52
17	12	51.9358	17.0189	S	0	11	0	2	0	1	104	0	10	0	0	0	128
17	13	51.8778	17.0844	S	18	3	1	20	1	12	1	1	18	1	0	0	76
18	1	51.9486	18.1322	S	46	34	0	5	0	14	26	0	9	0	0	0	134
18	2	51.9239	18.1172	S	18	25	38	36	3	3	11	15	9	0	0	0	158
18	3	51.9997	17.7892	O	5	3	0	6	0	6	10	0	6	0	0	0	36
18	4	51.9994	17.7978	S	39	22	0	21	0	8	27	0	16	0	0	0	133
18	5	51.9994	17.7872	O	43	30	0	24	3	6	8	0	9	1	0	0	124
18	6	51.9042	18.0017	S	13	4	0	7	0	17	32	1	58	0	0	0	132
18	7	51.9300	17.9233	O	30	30	0	16	1	10	24	0	9	0	0	0	120
18	8	51.9289	17.9192	S	44	7	0	17	0	28	6	1	24	0	0	0	127
18	9	51.9286	17.9442	O	77	10	0	12	1	54	7	0	6	0	0	0	167
18	10	51.9739	17.8692	O	1	9	0	54	11	1	5	0	21	4	0	0	106
18	11	51.9794	17.8600	S	0	1	1	82	6	0	0	0	36	0	0	0	126
18	12	51.9789	17.8583	O	0	6	14	45	58	0	1	2	8	8	0	0	142
18	13	51.9486	18.1306	O	60	33	0	8	1	9	11	0	4	0	0	0	126
18	14	51.9492	18.1303	S	20	80	0	12	0	5	31	0	7	0	0	0	155
18	15	51.9489	18.1311	O	53	28	0	4	0	16	18	0	10	0	0	0	129
19	1	51.9717	18.7856	S	0	4	0	28	0	28	0	0	0	0	22	0	82

Meridian No.	Population No.	Geographic coordinates		Habitat	Yellow					Pink					Brown		Total
					U	M	T	F	O	U	M	T	F	O	U	B	
19	2	51.9744	18.7908	O	0	25	0	25	0	33	0	0	0	0	8	0	91
19	3	51.9808	18.7972	O	0	0	1	54	0	49	0	0	0	0	22	0	126
19	4	52.1275	18.9983	S	0	6	1	2	0	22	0	0	0	0	1	0	32
19	5	52.1269	19.0056	O	9	25	17	23	0	4	12	9	24	1	0	0	124
19	6	51.9381	18.9914	O	0	52	11	19	0	1	34	8	10	0	0	0	135
19	7	51.8847	18.9614	O	0	58	8	8	0	9	36	3	3	0	0	0	125
19	8	51.8761	18.9328	S	0	54	4	38	4	0	23	0	4	0	0	0	127
19	9	51.8789	18.9308	O	0	10	0	11	1	1	42	1	37	0	0	0	103
19	10	51.8911	18.7994	O	0	16	0	54	0	44	5	0	2	0	7	0	128
19	11	51.0203	18.8997	S	0	0	0	5	0	0	10	0	22	0	5	0	42
19	12	52.0378	18.9064	O	0	0	73	26	7	0	0	24	12	2	0	0	144
19	13	52.0386	18.9056	S	0	1	43	35	7	2	0	21	18	2	0	0	129
19	14	51.9711	18.7969	S	0	8	0	30	0	40	0	0	0	0	46	0	124
20	1	51.9789	20.1711	S	0	9	9	5	2	42	12	17	12	0	20	0	128
20	2	51.9794	20.1711	O	0	4	12	8	0	33	18	23	15	1	12	0	126
20	3	51.9797	20.1719	S	0	3	10	9	3	28	17	20	31	0	8	0	129
20	4	51.9394	20.1564	O	0	7	19	7	0	21	19	30	12	1	8	2	126
20	5	51.9447	20.1547	S	0	9	12	12	2	19	29	21	25	2	13	0	144
20	6	51.1111	19.9297	S	1	18	0	3	1	55	36	3	7	0	0	0	124
20	7	52.1089	19.9294	O	1	27	8	7	1	35	31	16	8	2	0	0	136
20	8	52.1033	19.9222	O	42	19	0	32	0	5	0	0	1	0	24	1	124
20	9	52.0944	19.9403	S	29	32	7	7	1	12	23	7	14	0	1	0	133
20	10	52.0939	19.9406	O	22	23	8	16	0	10	25	11	14	1	0	0	130
20	11	52.0728	20.105	S	0	3	33	17	1	0	1	32	12	0	24	0	123
20	12	52.0686	20.1692	S	1	7	3	15	0	19	11	4	8	0	0	0	68
20	13	52.0717	20.1697	S	2	5	0	1	0	69	34	4	24	0	0	0	139
20	14	51.9472	20.1458	S	0	1	2	1	0	3	7	35	7	0	1	0	57
21	1	52.0283	20.9600	S	0	48	0	8	0	7	60	0	9	0	0	0	132
21	2	52.0297	20.9592	S	1	60	1	7	1	2	55	0	5	0	8	1	141
21	3	52.0297	20.9511	O	2	50	1	19	2	6	41	5	13	1	0	0	140
21	4	52.0289	20.9500	O	3	59	5	15	0	4	63	6	12	1	0	0	168
21	5	51.9786	20.8544	O	0	34	0	32	2	4	28	0	20	0	0	0	120
21	6	51.9783	20.8539	S	0	46	0	16	0	8	58	0	12	0	0	0	140
21	7	51.9556	20.8864	S	0	26	4	4	0	59	18	5	3	4	0	0	123
21	8	51.8911	20.8200	O	0	38	9	19	3	9	31	5	9	1	0	0	124
21	9	52.0092	20.9614	S	0	22	0	22	0	32	27	0	21	1	0	0	125
21	10	51.9625	21.0367	O	1	45	3	14	0	17	29	2	16	0	0	0	127
22	1	51.9711	21.9678	S	0	0	0	0	0	101	28	0	3	0	0	0	132
22	2	51.9708	21.9603	O	0	56	15	13	0	36	3	5	1	0	0	0	129
22	3	51.9653	21.9603	O	0	3	0	12	0	86	8	0	12	0	0	0	121
22	4	51.0183	21.9014	S	0	0	0	14	0	3	1	0	90	1	13	0	122
22	5	52.0308	21.8108	O	1	43	0	39	3	0	28	0	20	0	0	0	134
22	6	52.0692	21.8494	S	2	24	0	27	0	4	42	4	56	1	0	0	160
22	7	52.0083	21.92	S	0	1	0	3	0	79	4	0	44	1	0	0	132



Meridian No.	Population No.	Geographic coordinates		Habitat	Yellow					Pink					Brown		Total
					U	M	T	F	O	U	M	T	F	O	U	B	
22	8	52.0067	21.8539	O	0	52	0	0	0	0	46	0	0	0	0	0	98
22	9	52.0089	21.8506	S	0	44	0	2	0	0	96	0	1	0	0	0	143
22	10	52.0081	21.8483	O	0	23	0	1	0	3	109	0	1	0	0	0	137
22	11	52.0625	22.1925	S	0	9	9	18	0	36	22	20	33	0	0	0	147
22	12	51.9558	21.9594	O	0	0	5	7	2	41	0	26	44	11	0	0	136
22	13	51.9578	21.9689	O	0	23	39	4	0	0	16	45	6	1	0	0	134
22	14	51.9592	21.9678	S	0	20	26	11	2	2	31	30	6	0	4	0	132
22	15	51.9603	21.9692	S	1	37	11	19	2	7	38	5	18	0	0	0	138
23	1	52.0208	23.1333	S	0	0	0	0	0	0	72	0	37	2	0	0	111
23	2	52.0208	23.1364	S	0	0	0	0	0	34	28	1	69	2	0	0	134
23	3	52.0189	23.1733	O	0	0	0	0	0	62	0	12	19	1	0	0	94
23	4	51.9997	22.7833	S	43	0	0	0	0	81	1	6	12	0	0	0	143
23	5	51.9875	22.7850	S	0	0	3	2	0	23	21	47	25	1	0	0	122
23	6	51.9944	22.8047	O	14	0	0	1	0	68	17	19	5	0	0	0	124
23	7	51.9908	22.8019	O	46	26	0	9	0	40	9	0	6	0	0	0	136
23	8	51.9864	22.7928	S	0	2	0	3	0	46	37	26	18	0	0	0	132
23	9	51.9781	22.7972	S	10	0	0	0	0	58	18	5	8	0	0	0	99

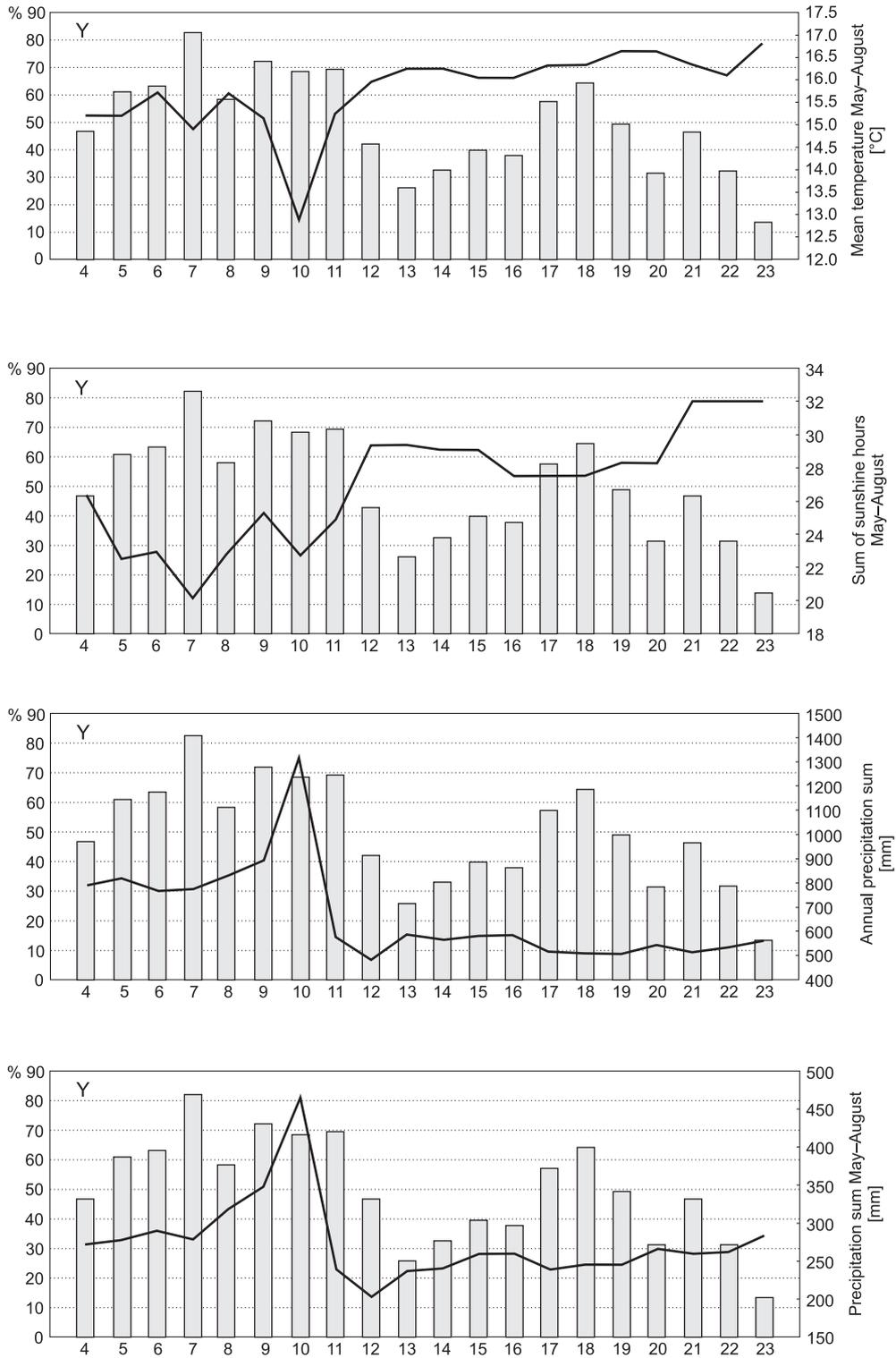
Appendix 2. The estimated climatic parameters for each plot (source: [www.fao.org/nr/climpag/locclim/locclim\\_en.asp](http://www.fao.org/nr/climpag/locclim/locclim_en.asp))

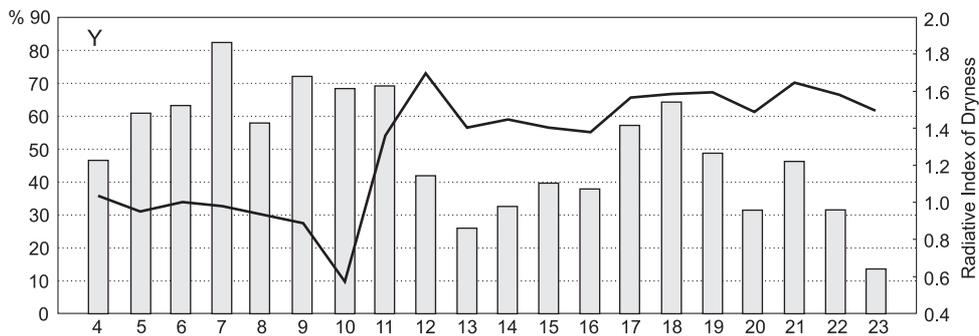
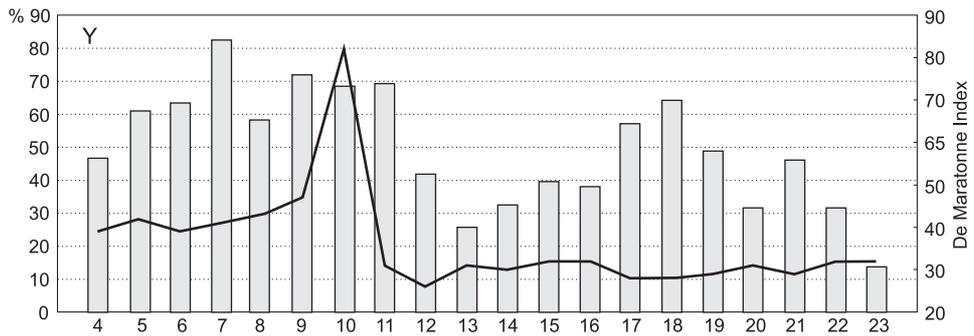
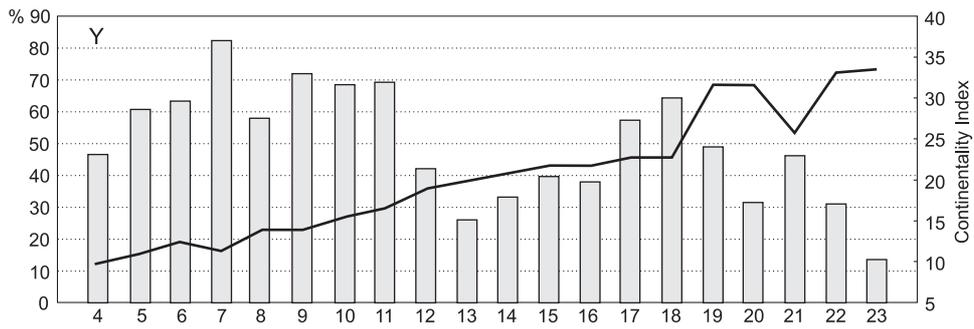
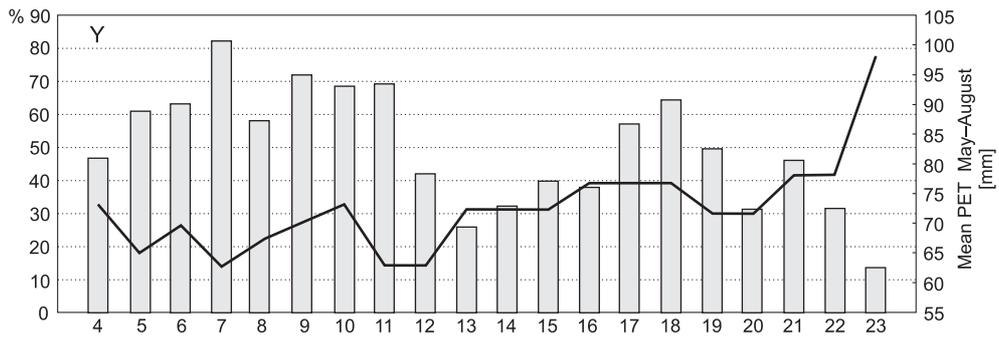
Plot	1	2	3	4	5	6	7	8	9	10
4	9.98	15.20	26.42	789	272	73.18	13.7	9.8	39	1.034
5	9.34	15.20	22.50	820	278	64.93	13.4	10.9	42	0.951
6	9.55	15.70	22.93	767	290	69.60	12.6	12.4	39	1.001
7	8.94	14.90	20.13	773	279	62.70	13.5	11.3	41	0.980
8	9.04	15.70	22.93	828	319	67.23	13.2	13.9	43	0.934
9	8.80	15.15	25.33	892	348	70.25	13.0	13.9	47	0.887
10	6.12	12.88	22.68	1317	466	73.15	11.4	15.4	82	0.571
11	8.32	15.23	24.88	575	240	62.85	11.2	16.5	31	1.359
12	8.58	15.95	29.38	484	202	62.85	12.9	18.9	26	1.696
13	8.65	16.25	29.38	585	237	72.35	12.9	19.9	31	1.404
14	8.53	16.25	29.08	564	240	72.35	12.6	20.8	30	1.448
15	8.21	16.05	29.08	582	260	72.35	12.6	21.7	32	1.404
16	8.21	16.05	27.52	582	260	76.75	12.6	21.7	32	1.380
17	8.18	16.33	27.52	515	239	76.75	13.1	22.7	28	1.565
18	8.18	16.33	27.52	508	245	76.75	13.1	22.7	28	1.587
19	7.49	16.63	28.30	508	245	71.55	13.0	31.6	29	1.595
20	7.49	16.63	28.30	545	266	71.55	13.4	31.6	31	1.488
21	7.74	16.35	32.05	515	260	78.13	13.4	25.6	29	1.647
22	6.92	16.10	32.05	533	262	78.13	13.4	33.1	32	1.588
23	7.43	16.83	32.05	562	284	98.35	13.1	33.5	32	1.494

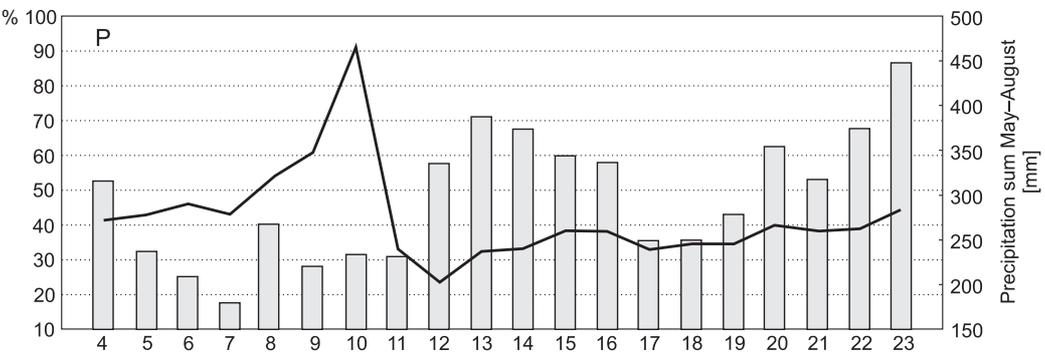
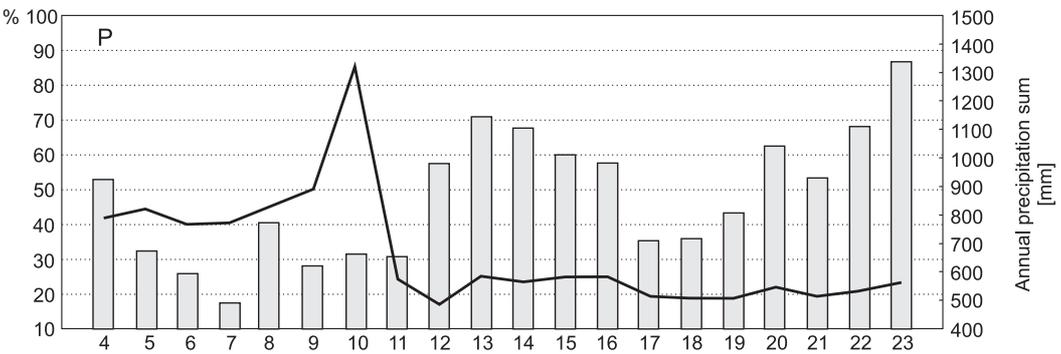
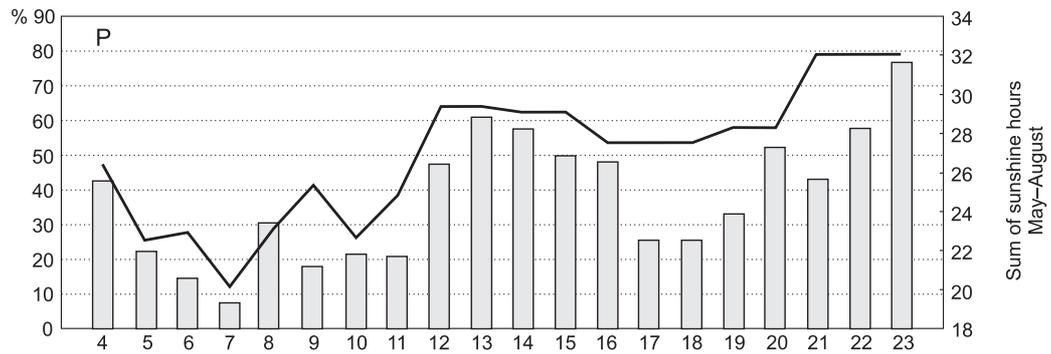
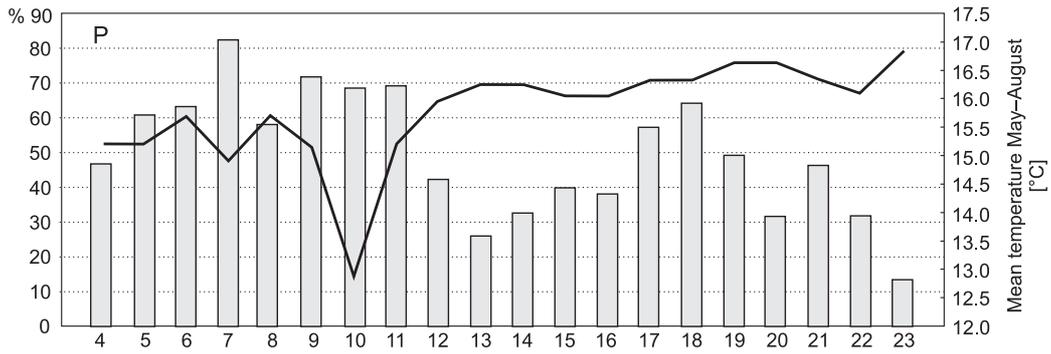
1. Mean annual temperature [C]
2. Mean temperature May–August [C]
3. Sum of sunshine hours May–August [h]
4. Sum of annual precipitation [mm]
5. Sum of precipitation May–August [mm]
6. Mean potential evapotranspiration May–August [mm]
7. Water vapour pressure May–August [hPa]
8. Gorchynski index of continentality
9. De Maratonne index
10. Radiative index of dryness

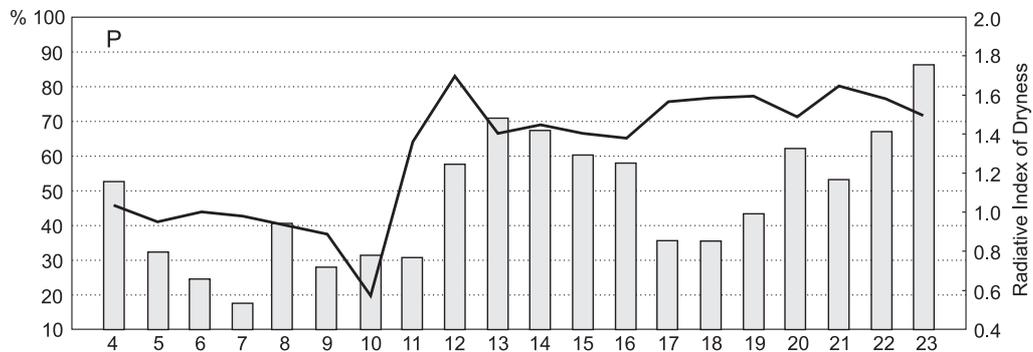
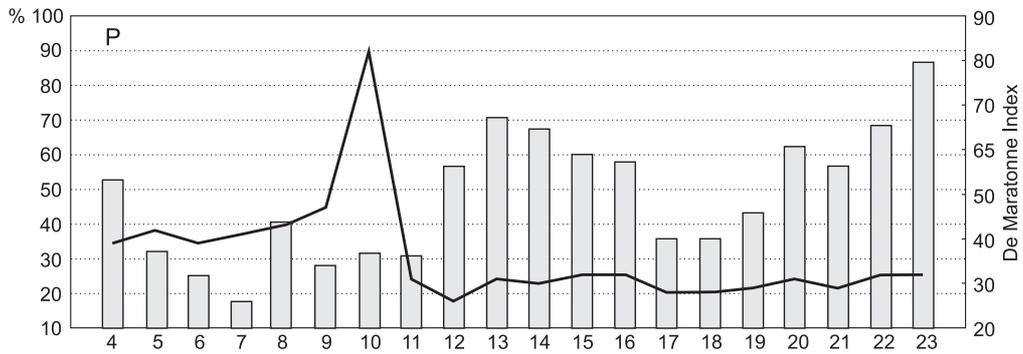
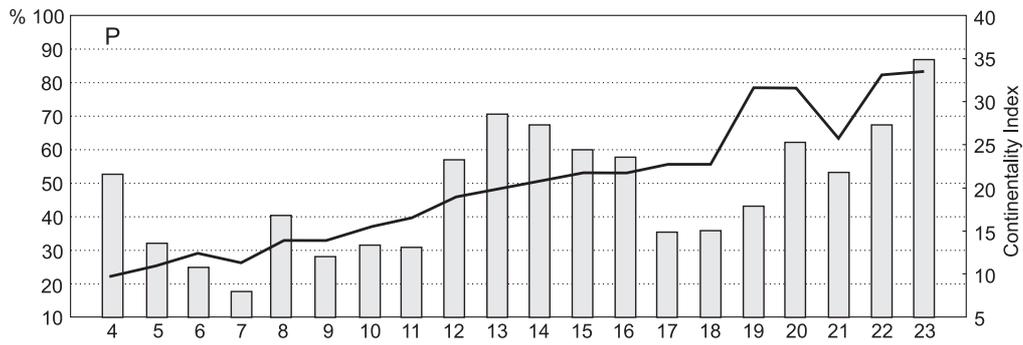
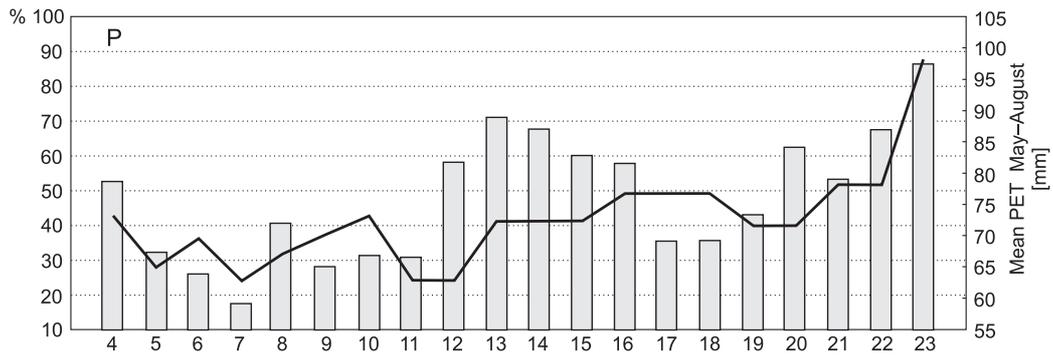


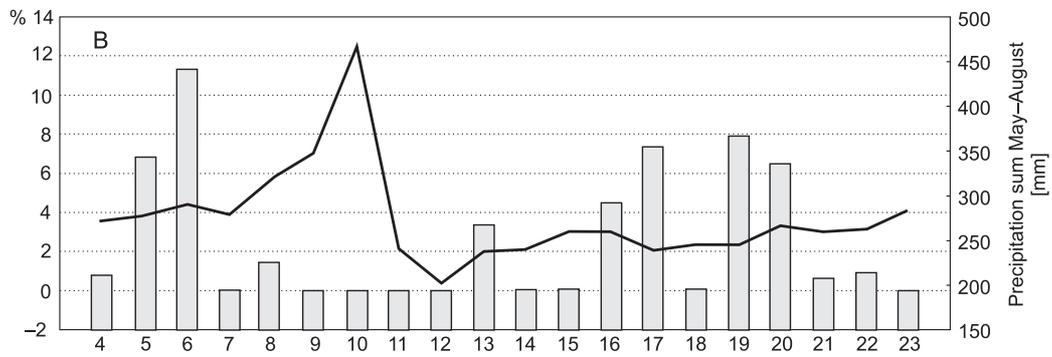
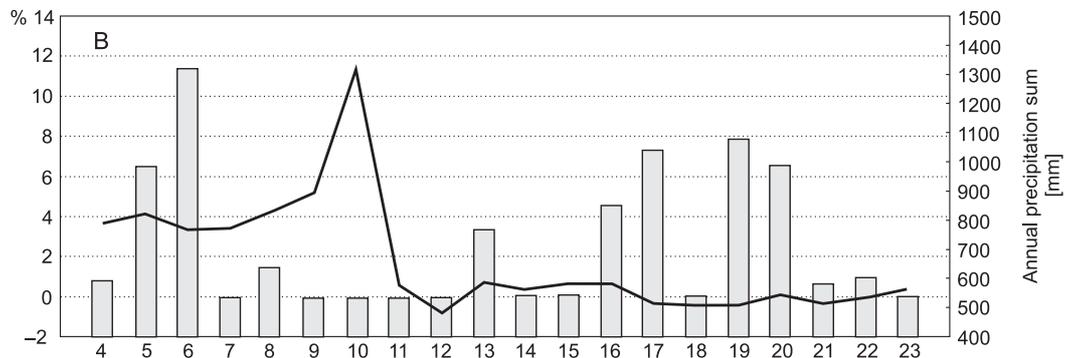
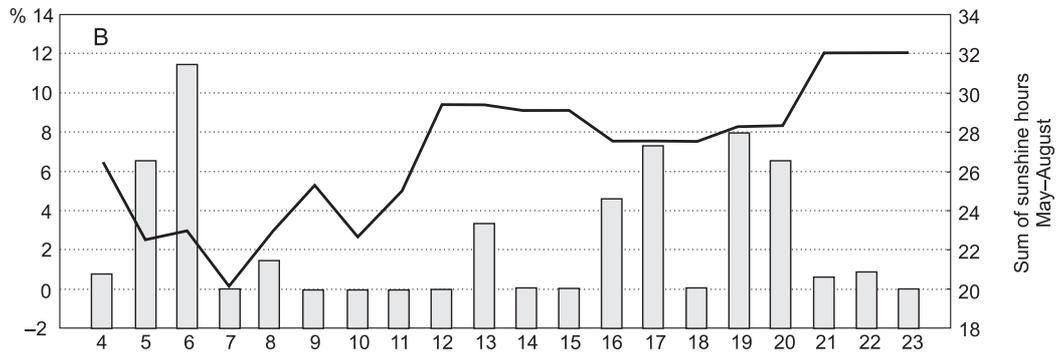
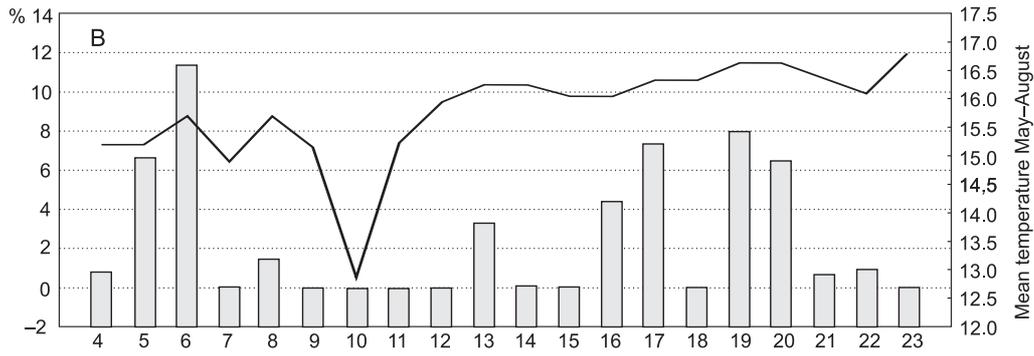
Appendix 3. Variation of morph frequencies and climatic parameters along the transect. Y – yellow, P – pink, B – brown, M – mid-banded, T – three-banded, F – five-banded, YeU – yellow effectively unbanded, IP – index of polymorphism, LD – linkage disequilibria between shell colour and the presence or absence of bands. 4–23 – plot numbers

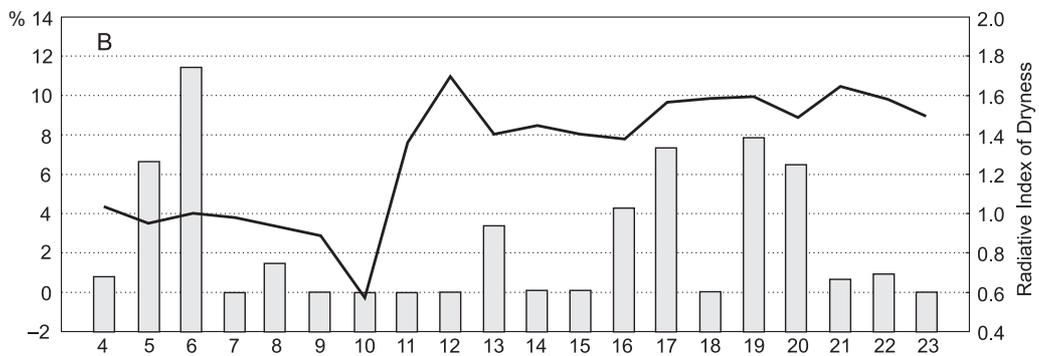
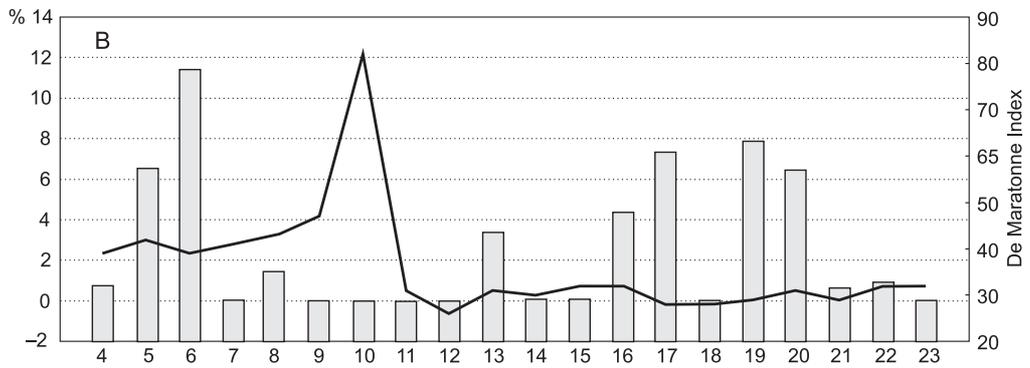
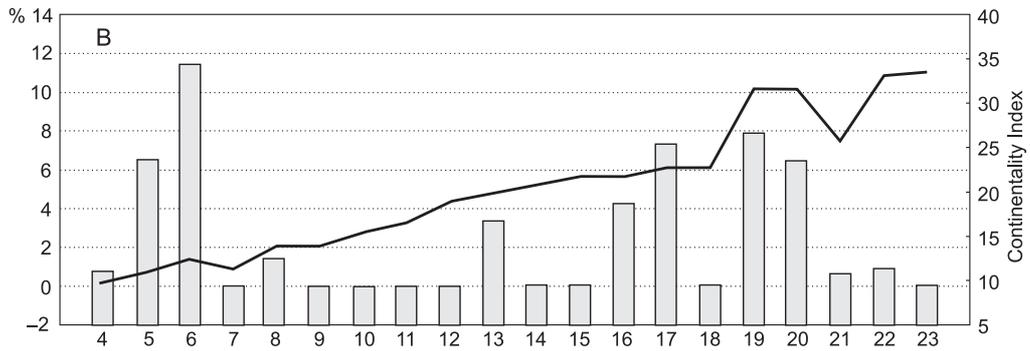
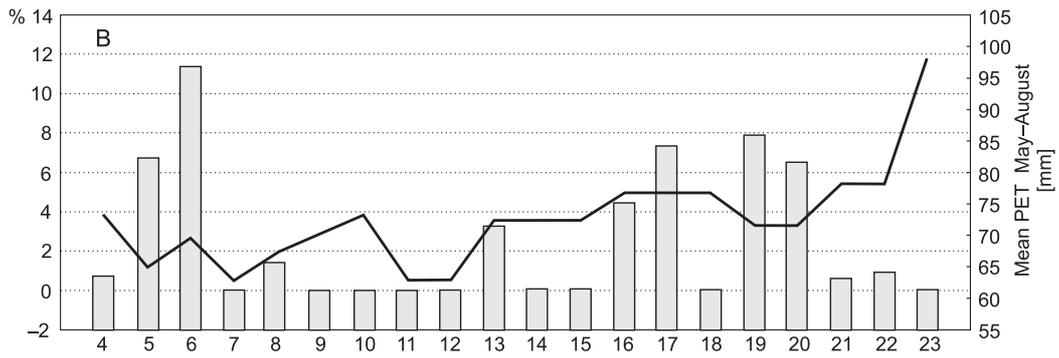


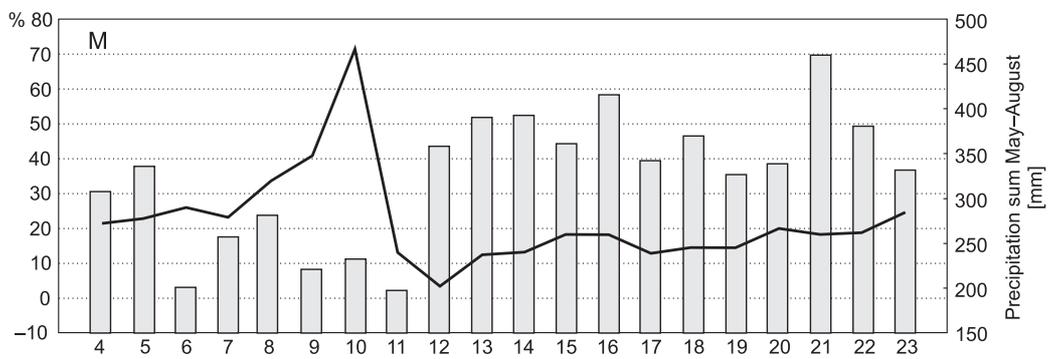
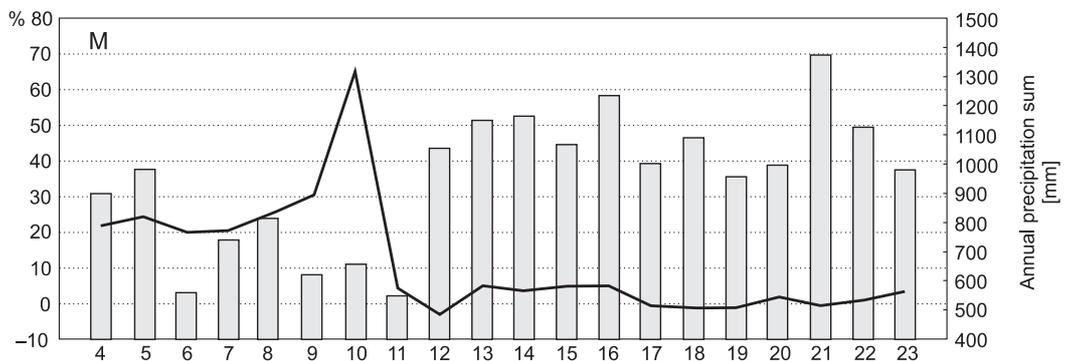
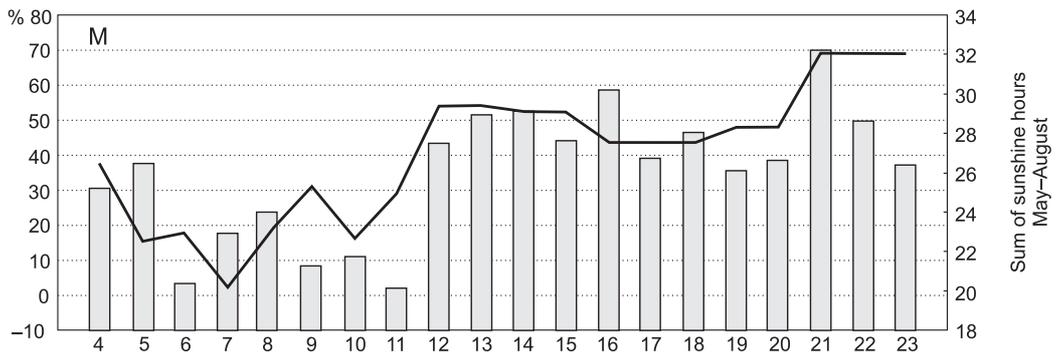
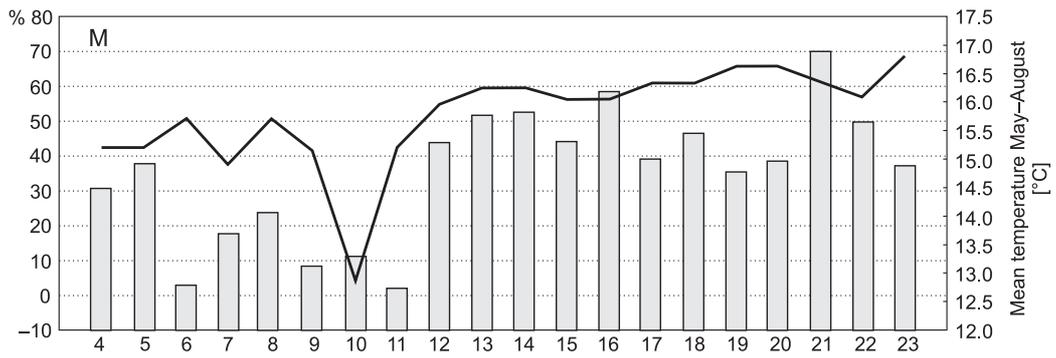


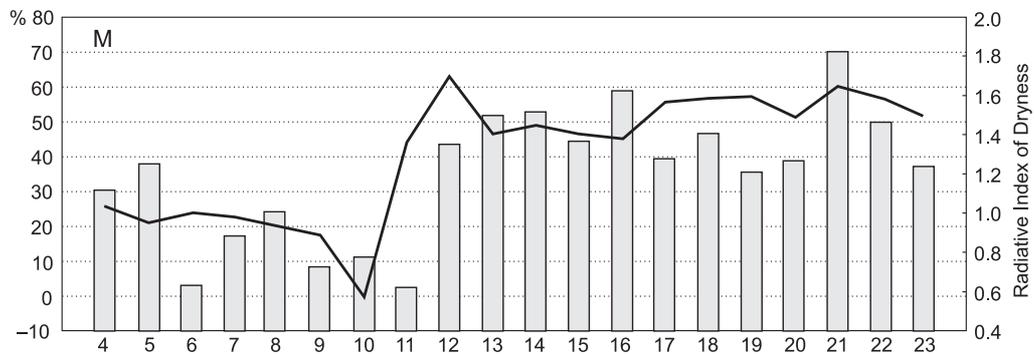
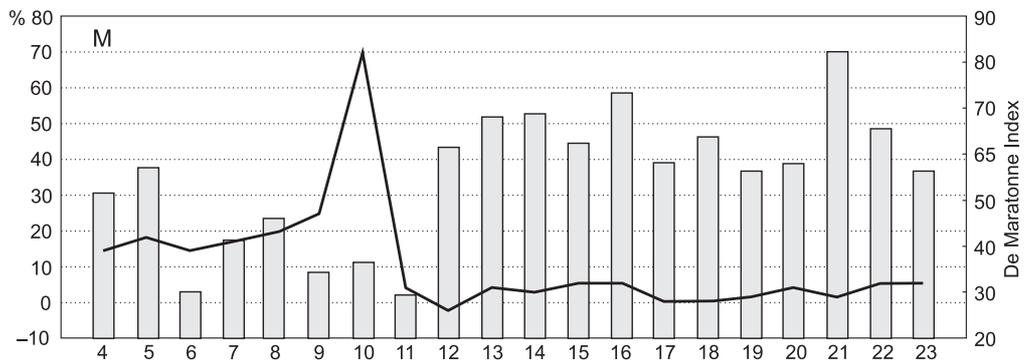
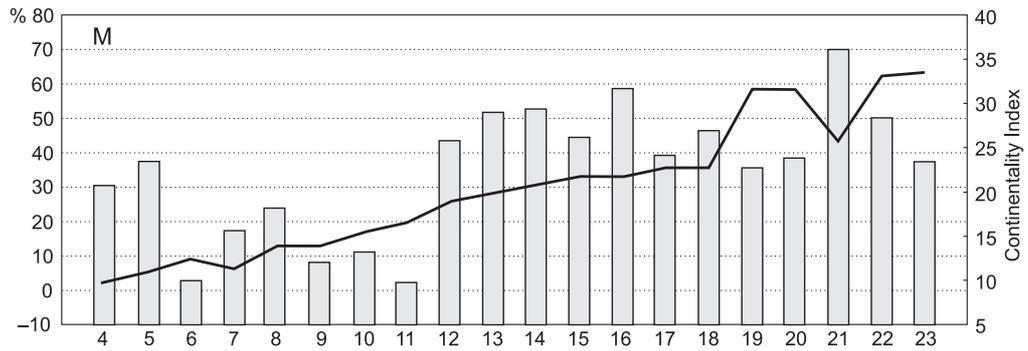
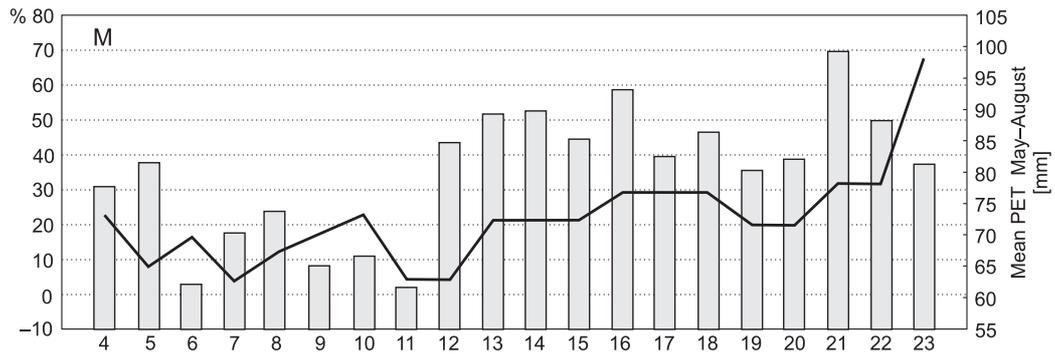


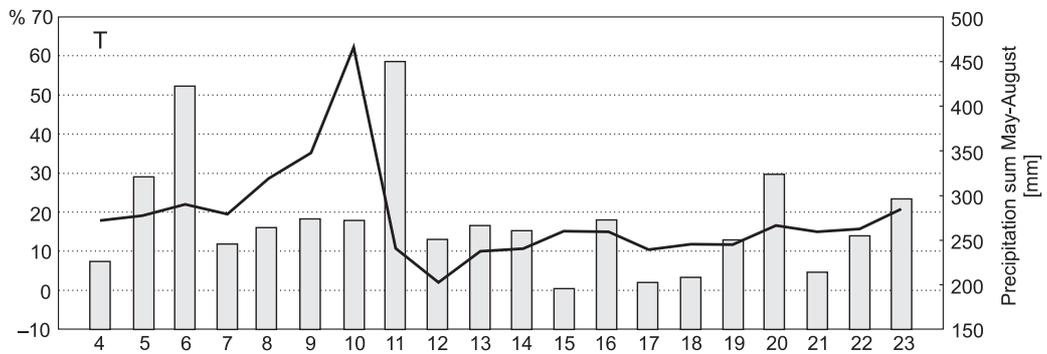
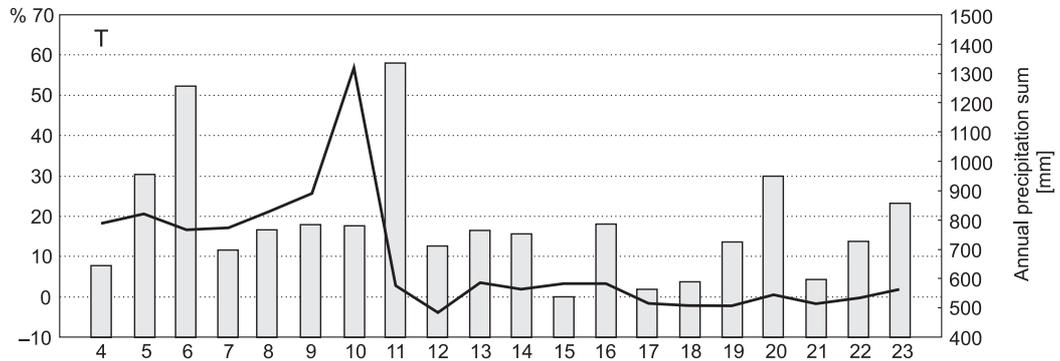
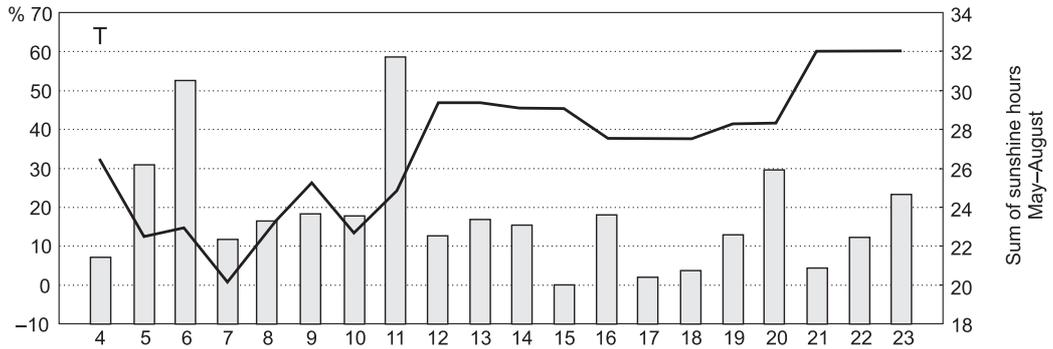
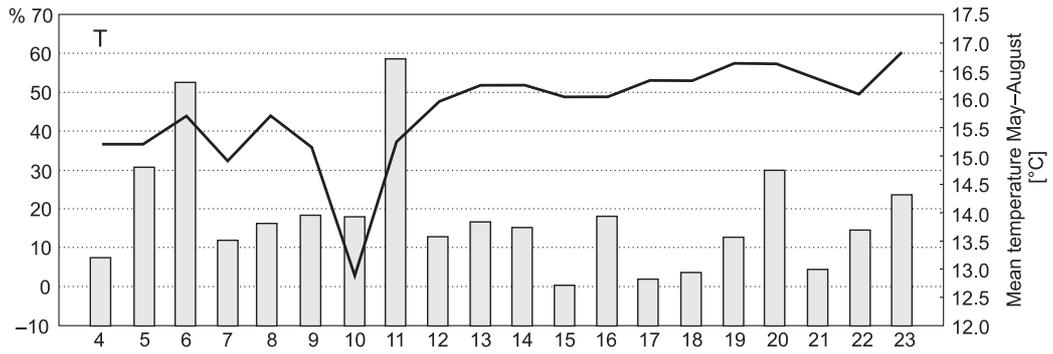


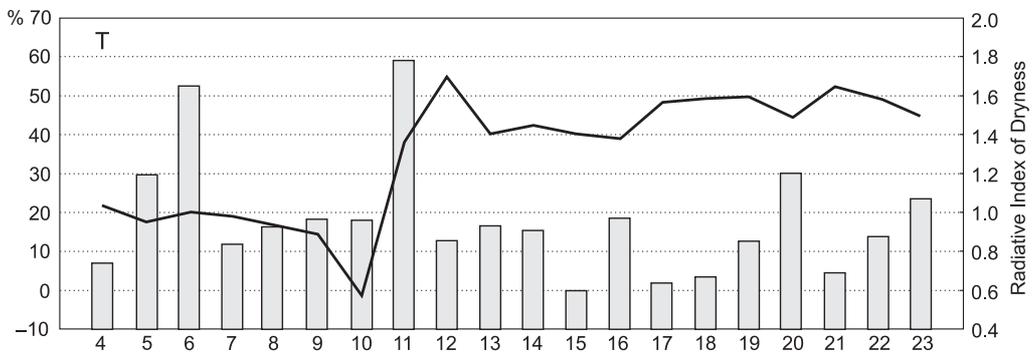
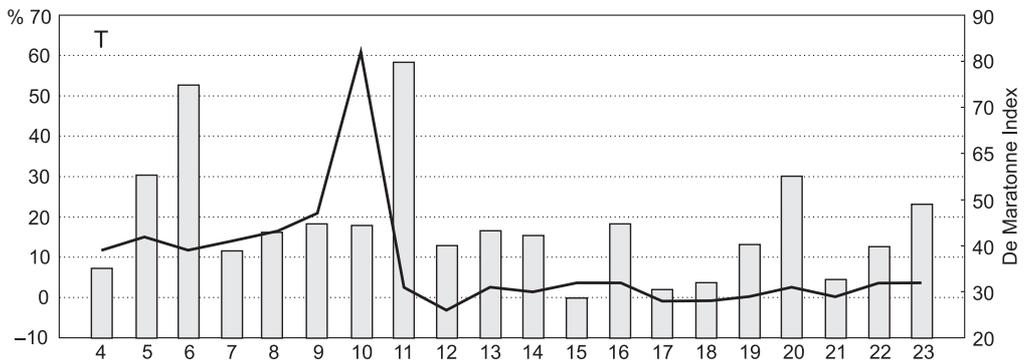
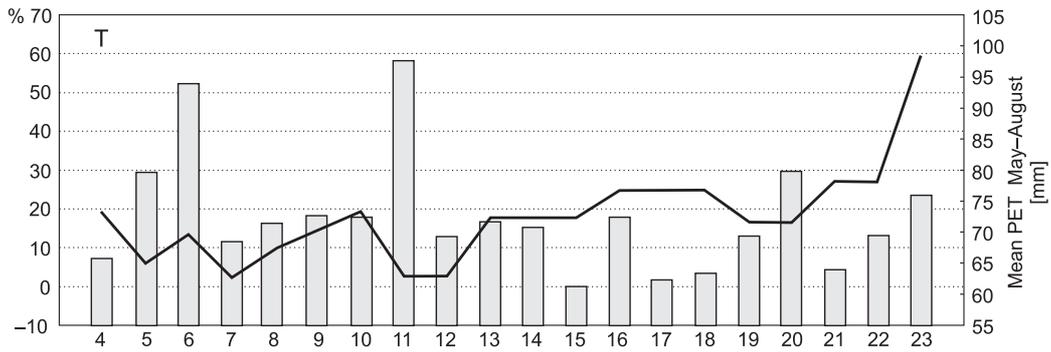
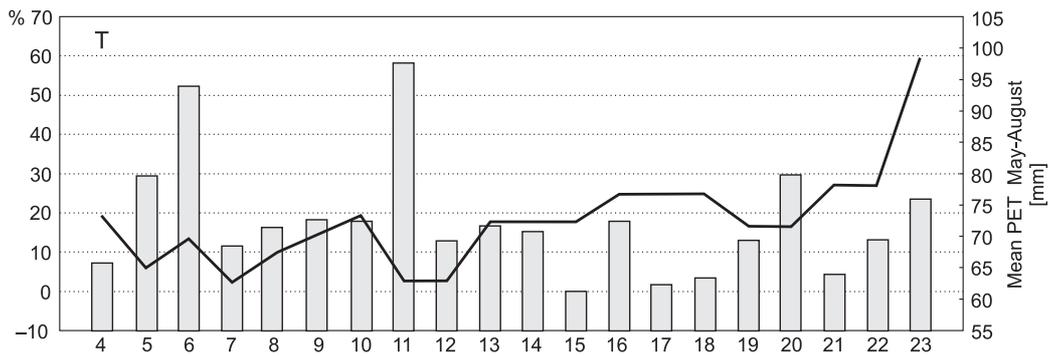


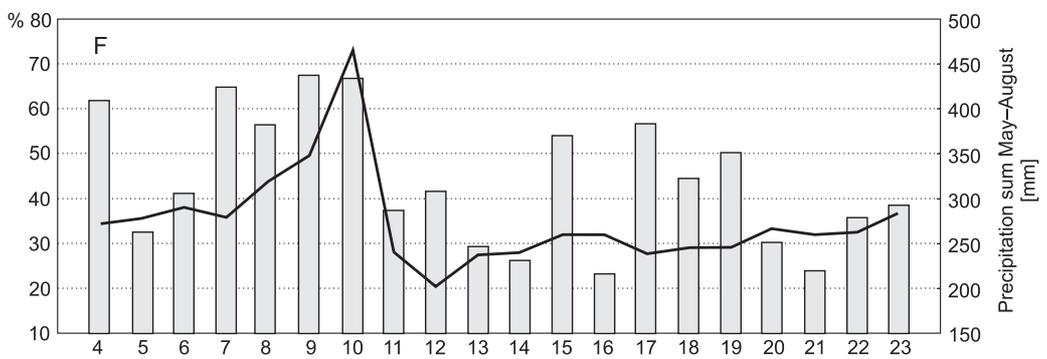
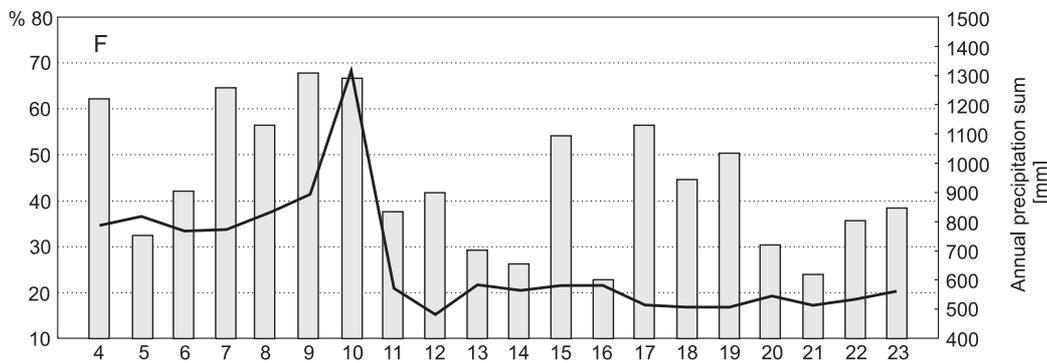
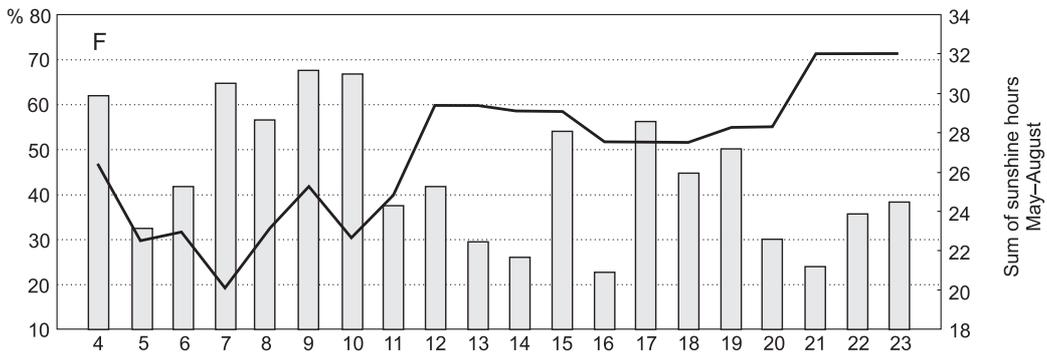
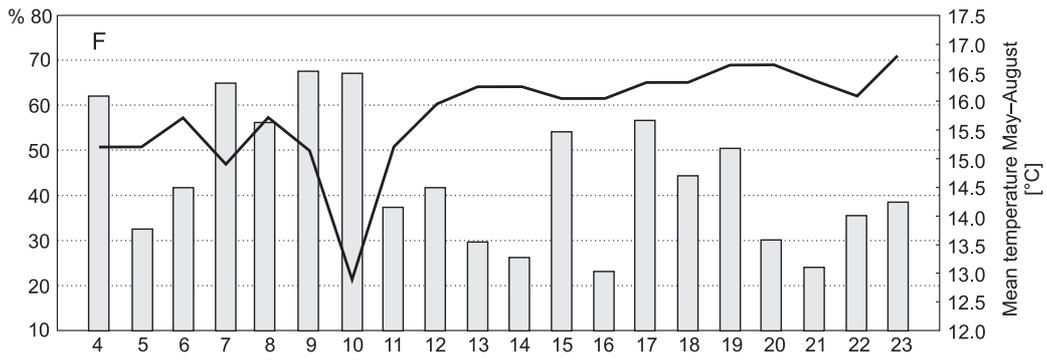


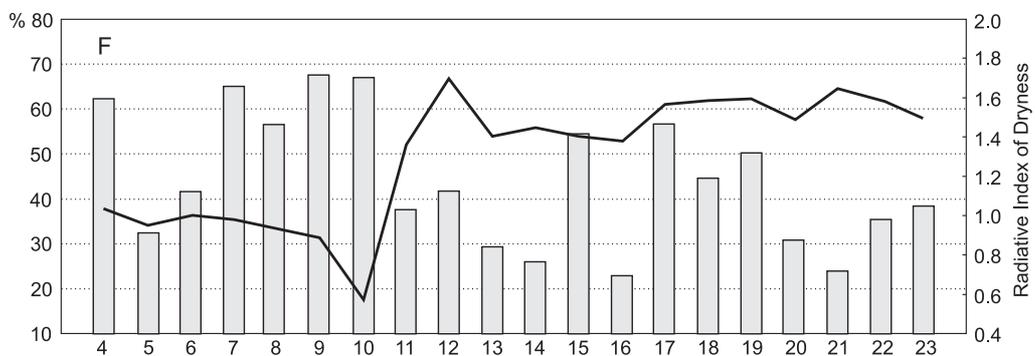
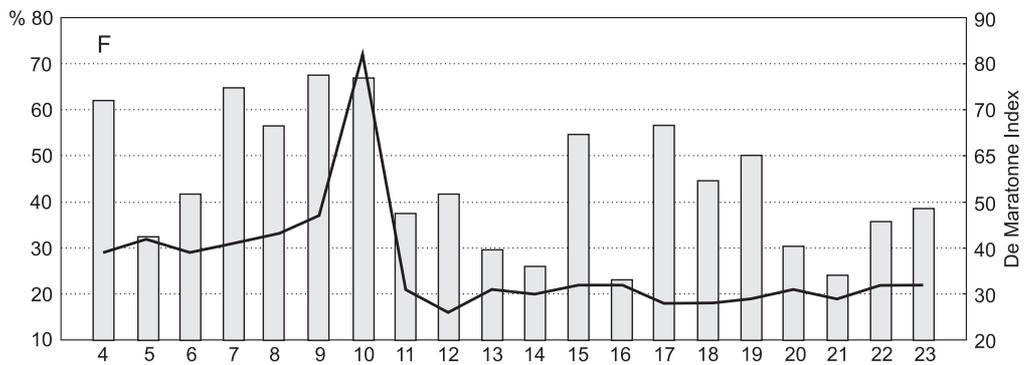
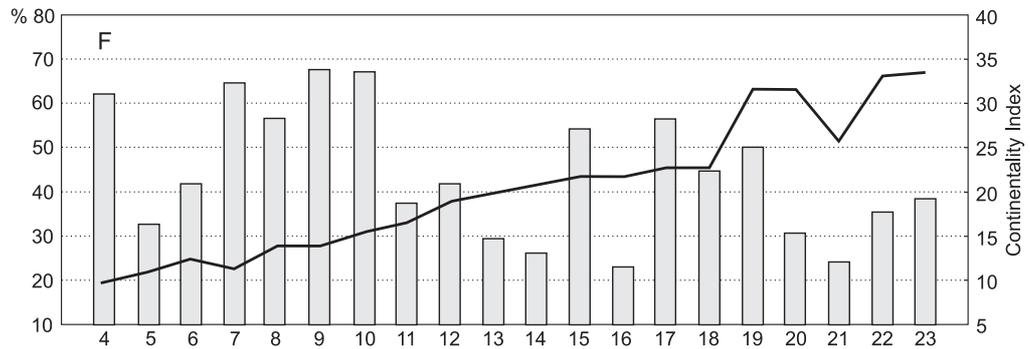
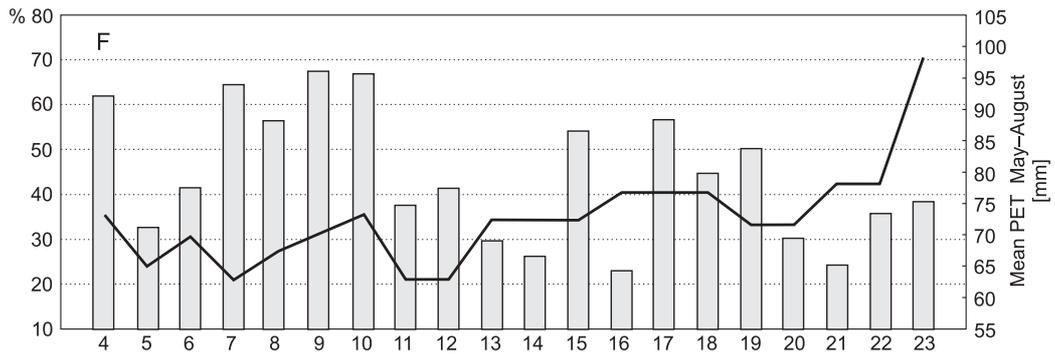


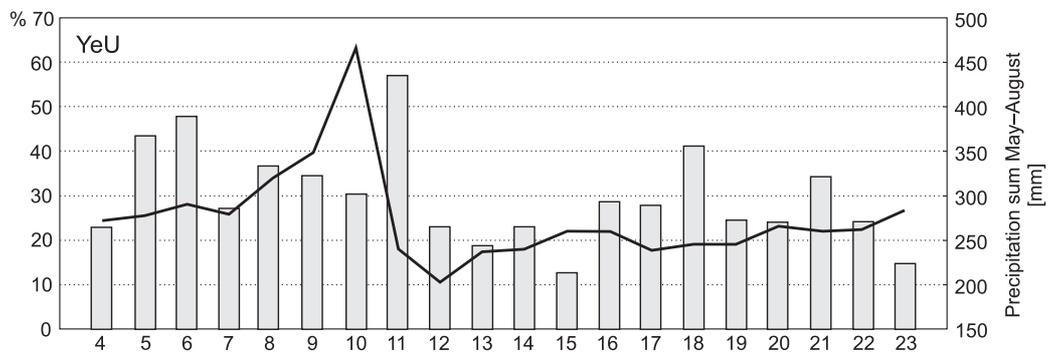
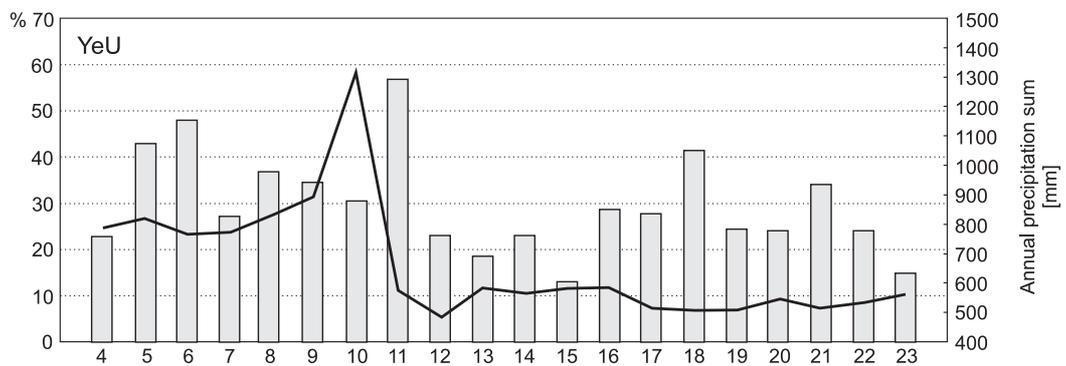
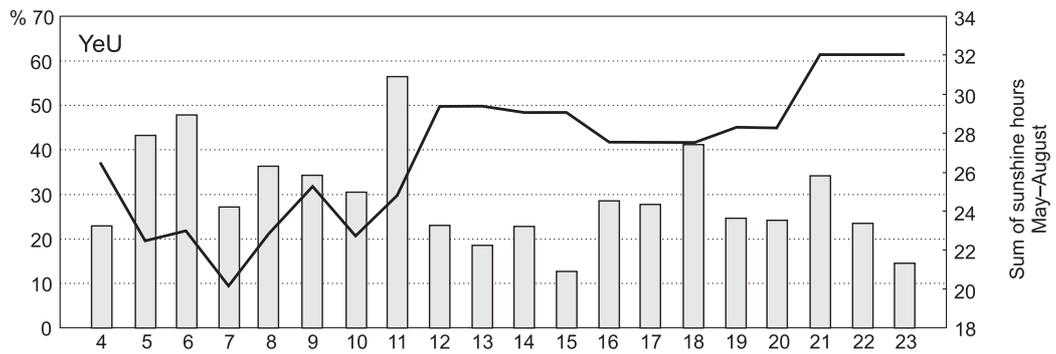
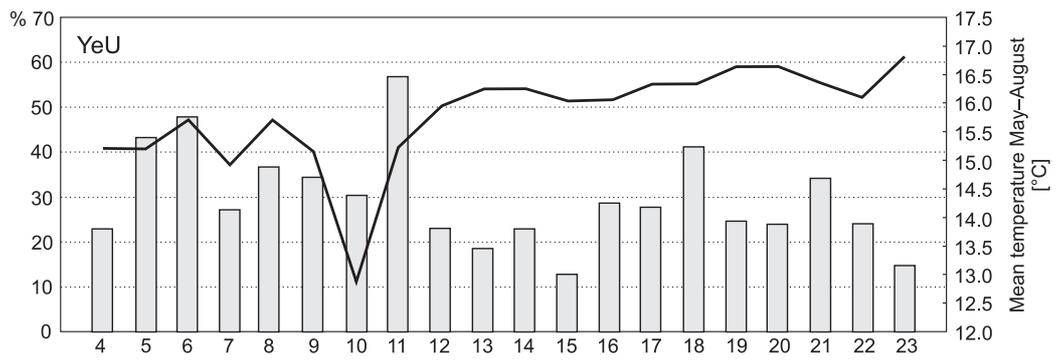


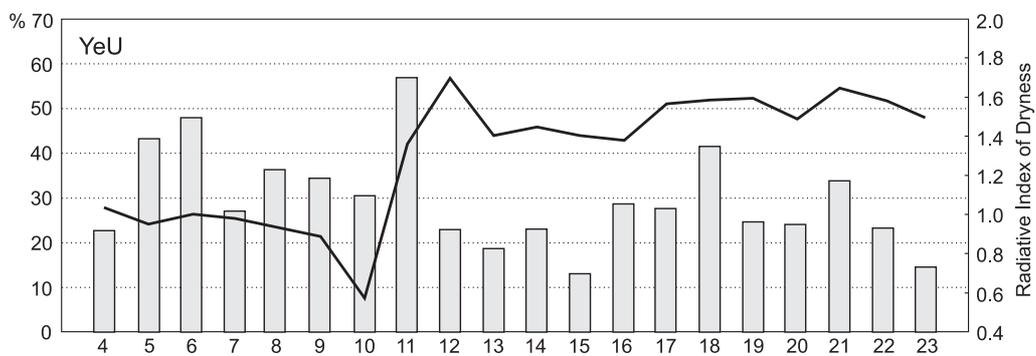
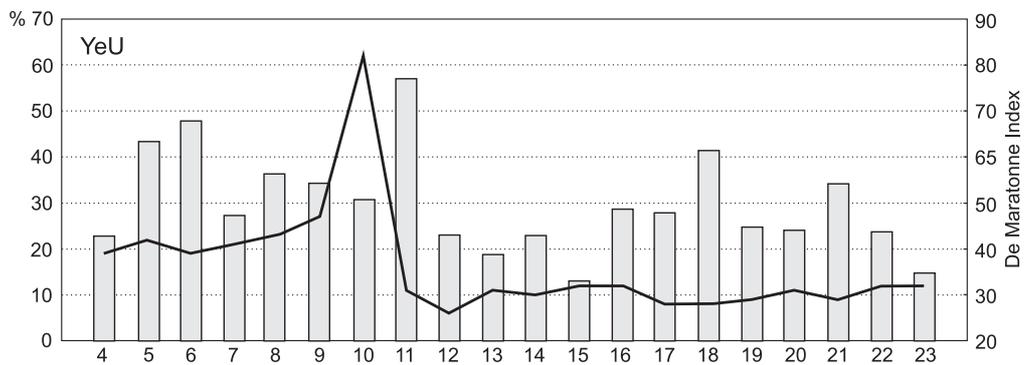
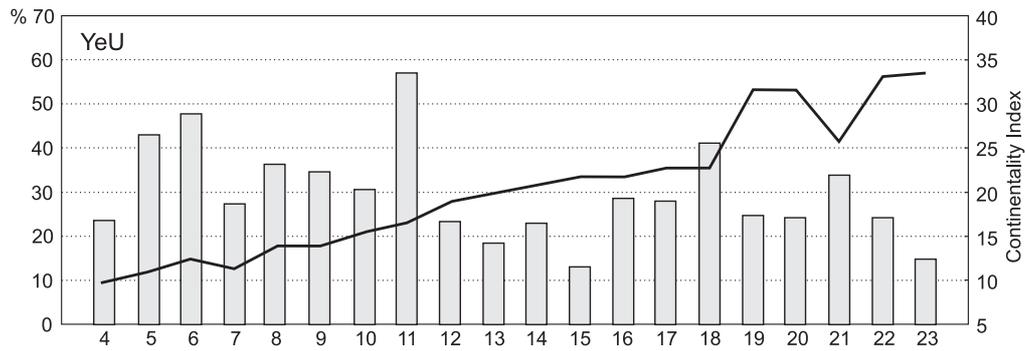
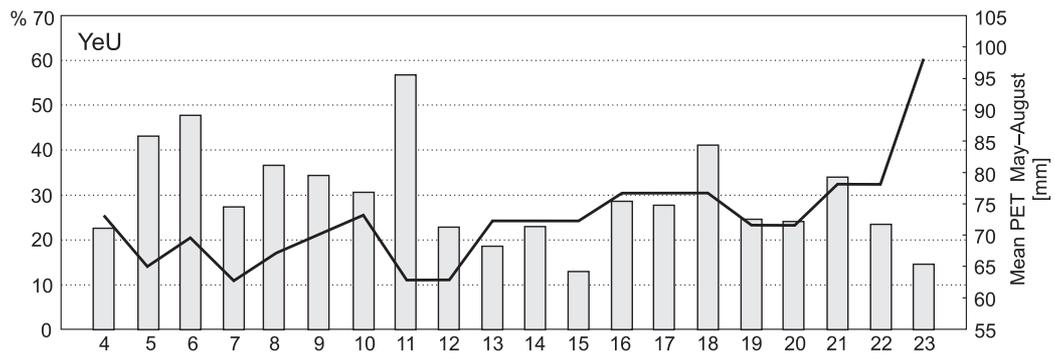


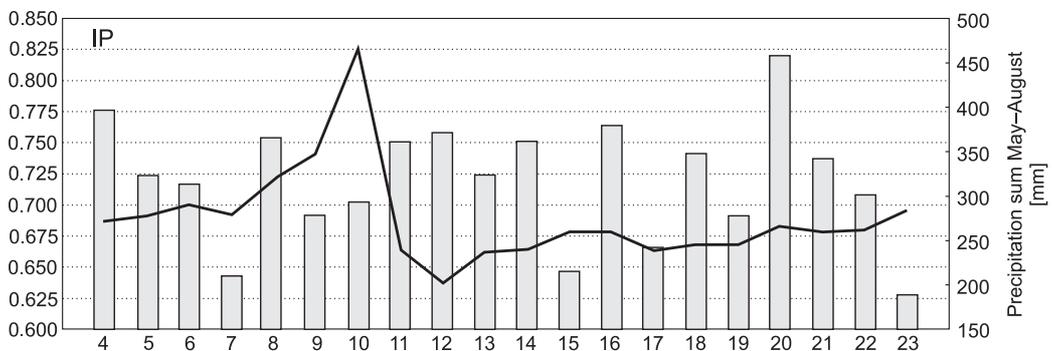
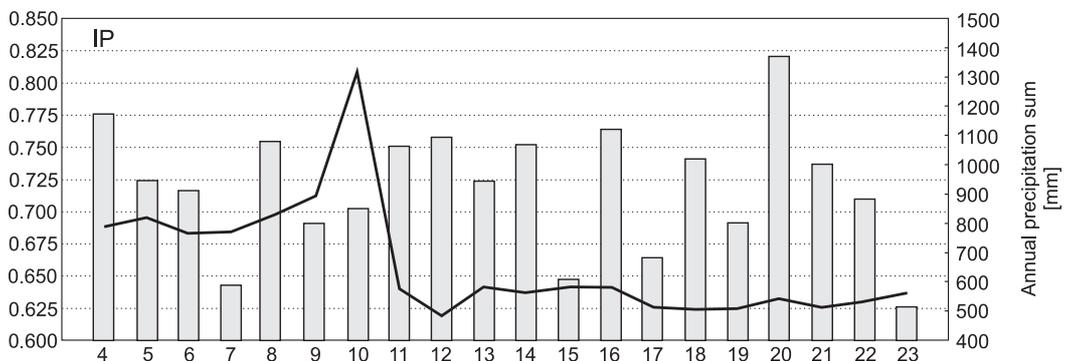
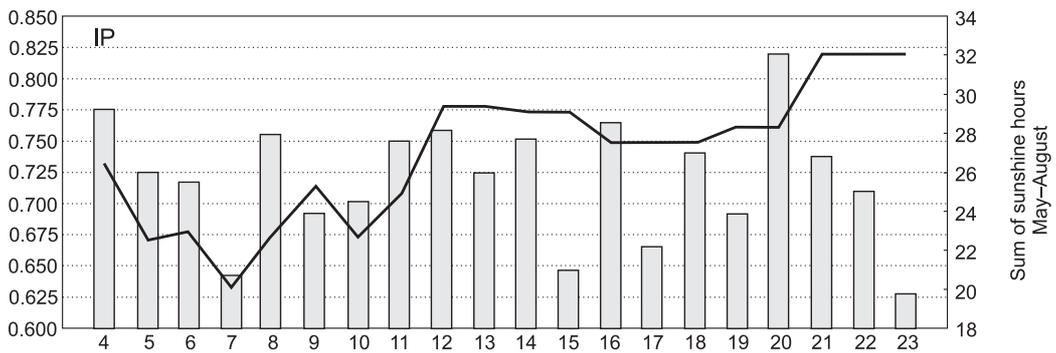
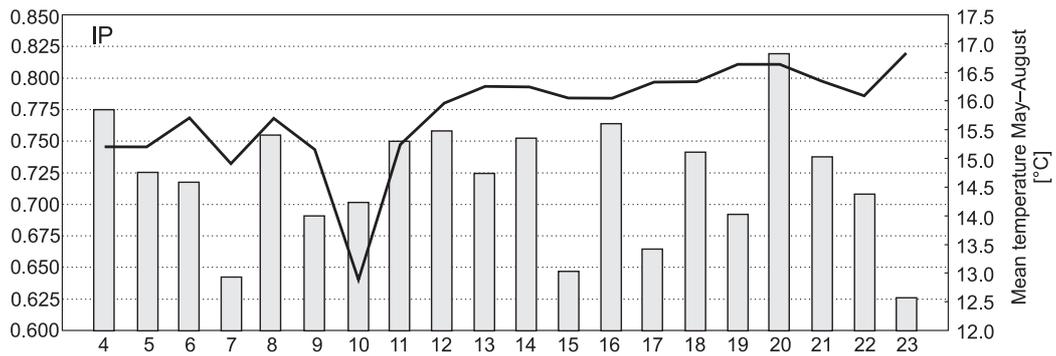


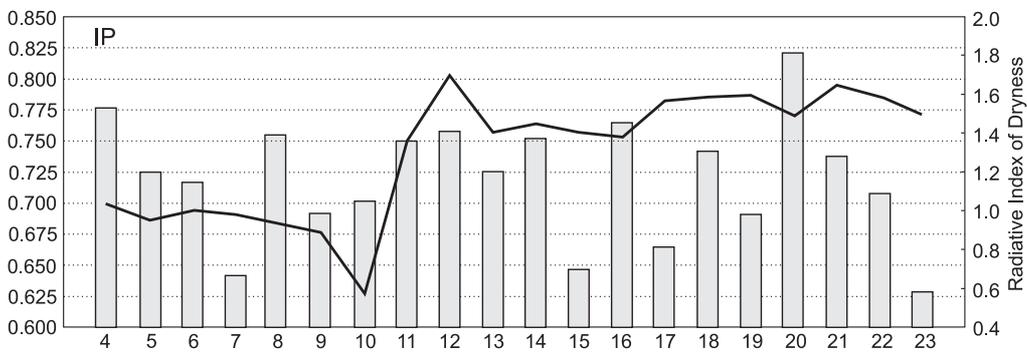
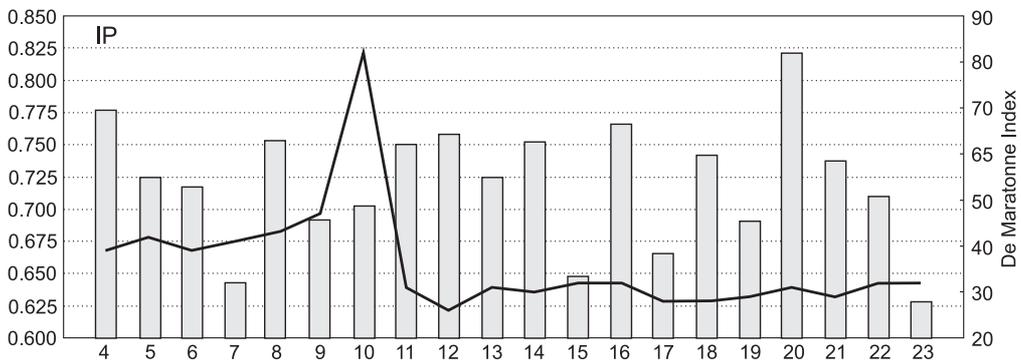
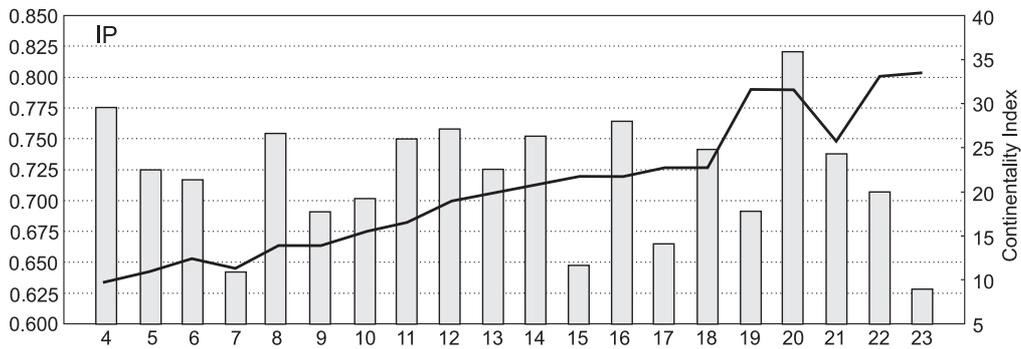
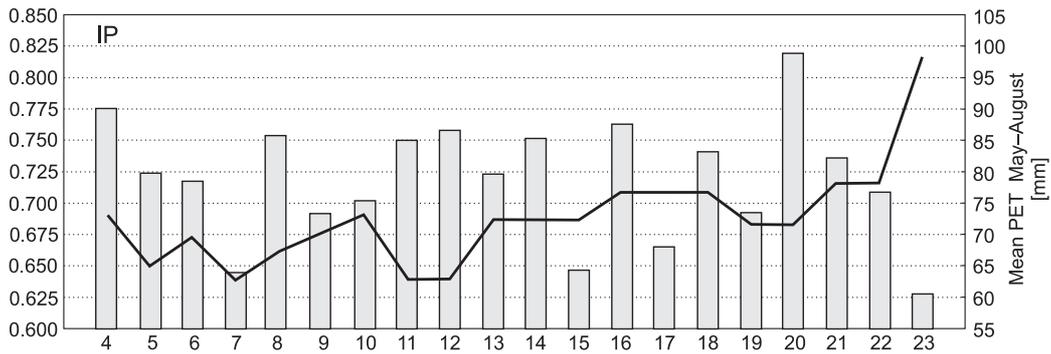


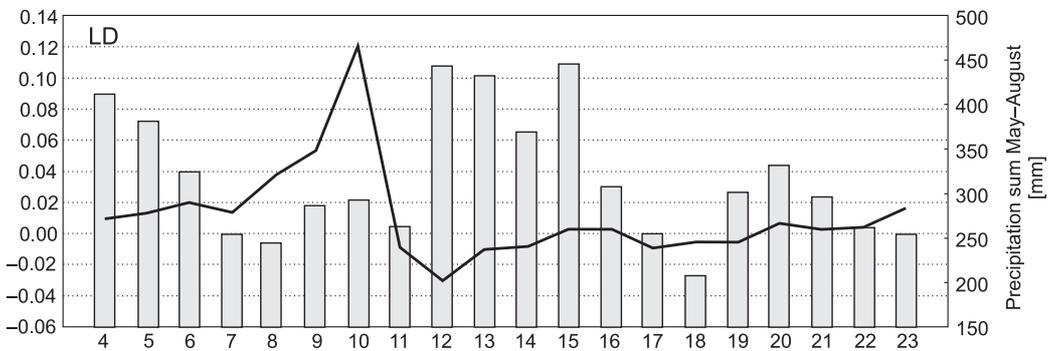
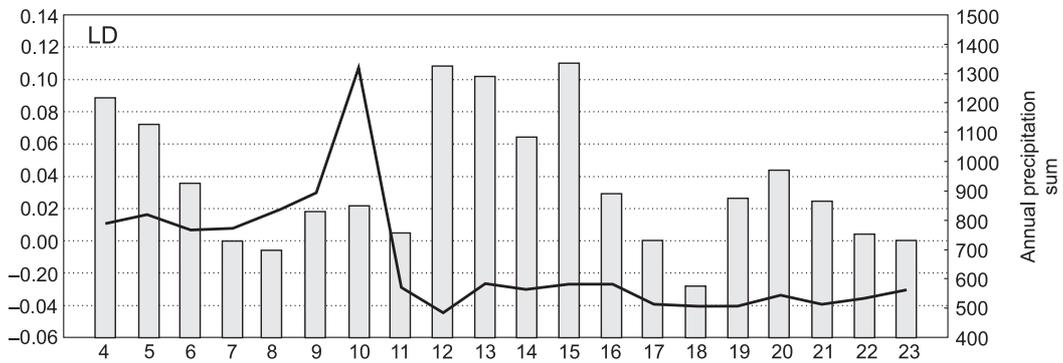
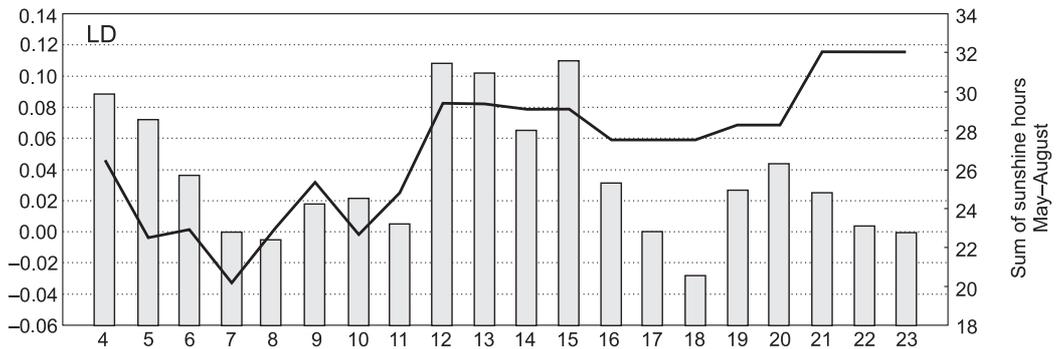
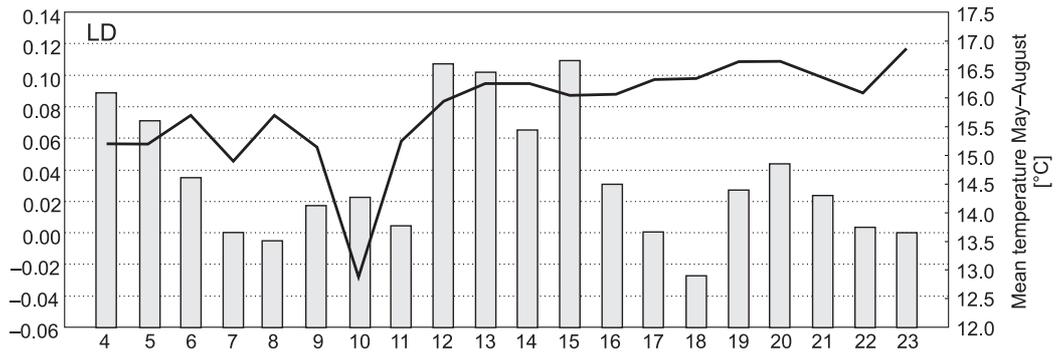


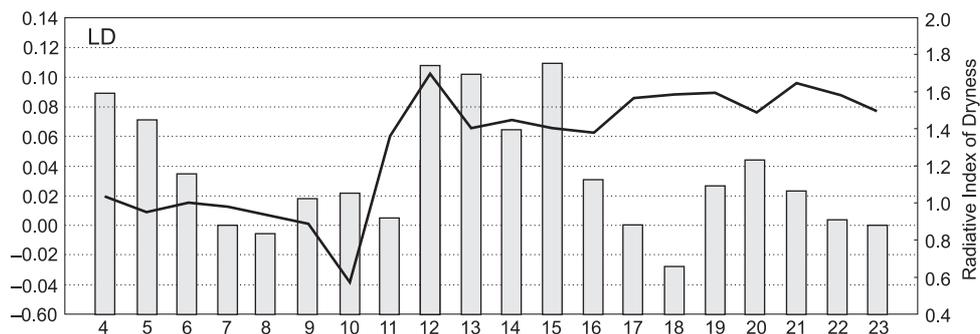
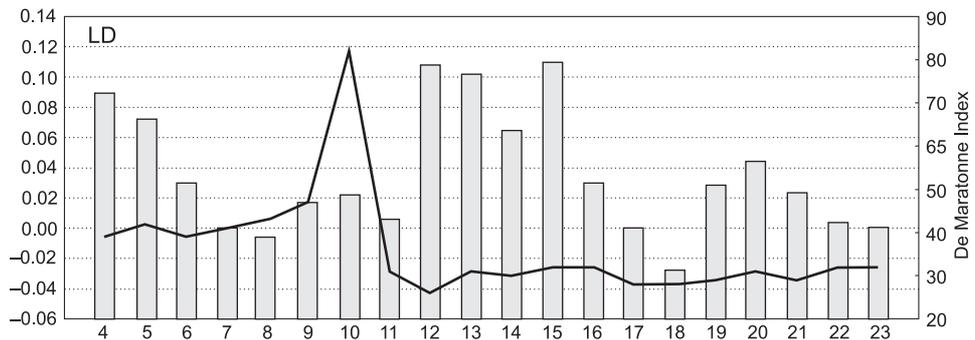
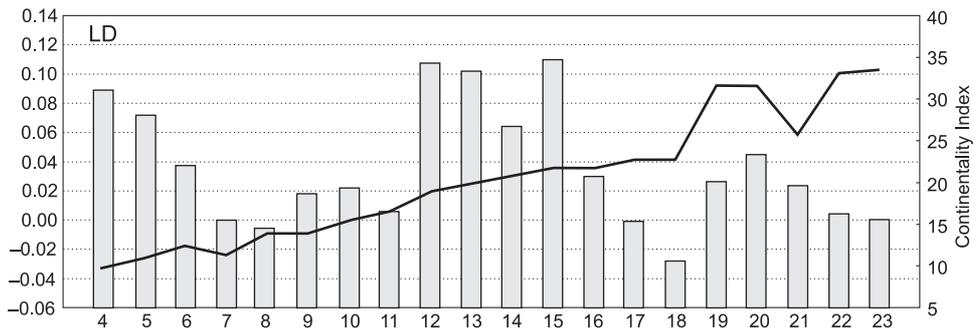
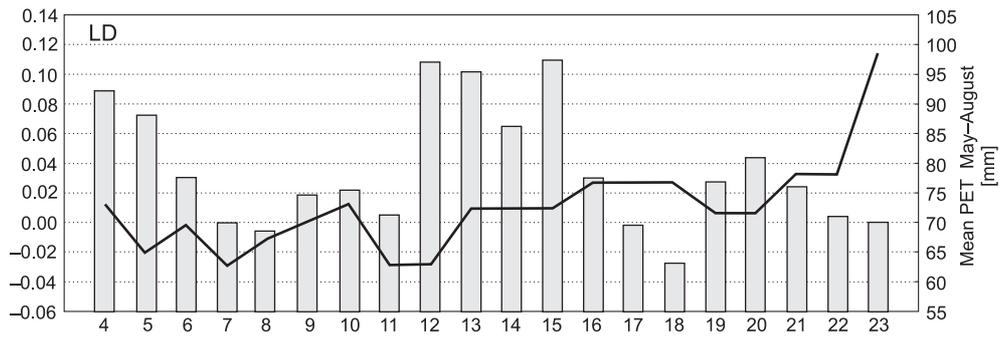














Appendix 4. Frequencies of band fusion in five-banded shells in plots 4–11 and 15–23. Habitat: O – open, S – shaded

Meridian No.	Population No.	Habitat	% Fused			Number of five-banded		
			All	Yellow	Pink	All	Yellow	Pink
4	2	O	7.5	8.3	6.3	40	24	16
4	4	O	10.2	12.2	0.0	49	41	8
4	5	O	11.5	7.1	21.1	61	42	19
4	6	S	60.0	54.5	64.3	50	22	28
4	7	S	64.7	64.7	—	0	17	17
4	8	S	37.8	33.3	41.7	45	21	24
4	9	O	13.3	16.7	0.0	15	12	3
4	10	S	61.1	57.1	63.6	18	7	11
4	11	S	16.7	7.7	40.0	18	13	5
5	5	O	15.0	12.5	25.0	20	16	4
5	6	S	5.0	0.0	11.1	20	11	9
5	7	O	25.0	16.7	50.0	8	6	2
5	8	O	39.5	37.8	100.0	38	37	1
5	9	O	0.0	0.0	—	6	6	0
5	10	S	0.0	0.0	0.0	25	19	6
5	11	S	42.9	33.3	100.0	14	12	2
5	12	O	26.1	11.8	66.7	23	17	6
5	13	O	25.0	33.3	0.0	24	18	6
5	14	O	13.6	13.6	—	22	22	0
5	15	S	58.8	50.0	71.4	34	20	14
6	1	S	15.0	15.0	—	20	20	0
6	2	S	0.0	0.0	—	14	14	0
6	3	O	31.3	33.3	0.0	16	15	1
6	4	O	6.7	7.1	0.0	15	14	1
6	5	O	15.4	22.2	0.0	26	18	8
6	6	S	33.3	33.3	—	9	9	0
6	7	O	28.6	25.0	50.0	14	12	2
6	8	O	13.0	9.4	18.2	54	32	22
6	9	S	23.9	23.9	—	46	46	0
6	10	O	0.0	0.0	0.0	8	5	3
6	11	O	0.0	0.0	—	2	2	0
7	1	O	45.5	40.0	100.0	11	10	1
7	2	O	43.1	43.1	—	58	58	0
7	3	O	58.7	58.8	58.3	46	34	12
7	4	O	48.6	48.6	—	72	72	0
7	5	S	72.2	70.9	81.8	97	86	11
7	6	S	60.5	63.9	42.9	43	36	7
7	7	S	65.4	68.2	50.0	26	22	4
7	8	O	37.7	35.9	39.5	77	39	38
8	1	O	37.5	37.5	—	16	16	0
8	2	S	84.3	80.0	86.1	51	15	36
8	3	O	50.0	30.0	57.7	36	10	26
8	4	O	36.8	10.0	42.6	57	10	47
8	5	O	55.0	50.0	66.7	40	28	12

Meridian No.	Population No.	Habitat	% Fused			Number of five-banded		
			All	Yellow	Pink	All	Yellow	Pink
8	6	S	4.0	0.0	16.7	25	19	6
8	7	O	0.0	0.0	—	5	5	0
8	8	S	90.0	100.0	88.9	10	1	9
8	9	O	75.0	50.0	100.0	4	2	2
8	10	O	27.8	20.7	57.1	36	29	7
9	1	S	63.3	63.6	62.5	30	22	8
9	2	S	37.0	30.6	50.0	73	49	24
9	3	O	28.8	25.6	35.0	59	39	20
9	4	S	27.9	29.3	20.0	68	58	10
9	5	O	47.4	47.4	—	19	19	0
9	6	O	54.8	25.0	65.2	31	8	23
9	7	S	48.8	48.7	50.0	43	39	4
9	8	S	55.2	50.0	80.0	29	24	5
9	9	O	52.0	51.1	60.0	50	45	5
10	1	O	46.5	45.5	50.0	71	55	16
10	2	S	28.1	27.1	33.3	57	48	9
10	3	S	77.5	77.8	76.6	40	27	13
10	4	O	46.8	51.4	42.5	77	37	40
10	5	S	58.8	59.2	58.1	102	71	31
10	6	O	63.9	63.9	—	36	36	0
10	7	O	60.9	61.5	60.0	46	26	20
10	8	O	59.2	59.2	—	49	49	0
10	9	S	63.3	60.3	67.3	120	68	52
10	10	S	57.7	56.9	61.5	78	65	13
10	11	S	76.5	71.4	84.6	34	21	13
11	1	O	33.3	25.0	40.0	9	4	5
11	3	O	42.9	50.0	16.7	28	22	6
11	4	S	50.0	50.0	50.0	32	22	10
11	5	S	4.2	4.8	0.0	24	21	3
11	6	S	38.2	38.5	37.5	34	26	8
11	7	S	36.4	40.0	0.0	11	10	1
11	8	S	83.9	84.8	82.6	56	33	23
11	9	S	63.0	54.2	72.7	46	24	22
11	10	O	39.0	36.0	43.8	41	25	16
11	11	O	33.3	40.0	0.0	6	5	1
11	12	O	50.0	50.0	—	8	8	0
11	13	O	78.6	78.6	—	14	14	0
15	2	S	36.7	23.1	40.4	60	13	47
15	4	O	25.6	22.2	33.3	39	27	12
15	7	S	50.7	46.0	64.7	67	50	17
15	8	O	27.5	24.7	80.0	102	97	5
15	9	S	64.2	54.8	77.3	53	31	22
15	10	O	4.9	4.9	—	61	61	0
15	11	O	7.4	0.0	10.5	27	8	19



Meridian No.	Population No.	Habitat	% Fused			Number of five-banded		
			All	Yellow	Pink	All	Yellow	Pink
15	12	S	33.3	33.3	33.3	9	3	6
15	13	S	35.3	37.5	0.0	17	16	1
16	1	O	69.0	69.0	—	42	42	0
16	7	S	69.4	60.0	84.2	49	30	19
16	8	O	45.5	33.3	50.0	11	3	8
16	9	S	41.7	25.0	50.0	12	4	8
16	10	O	40.6	33.3	41.4	32	3	29
16	11	S	73.8	0.0	75.6	42	1	41
16	12	O	0.0	0.0	—	5	5	0
16	13	O	7.7	14.3	5.3	26	7	19
17	9	O	54.0	40.9	58.5	87	22	65
17	10	O	5.9	5.9	—	34	34	0
17	11	O	10.5	10.5	—	19	19	0
17	12	S	8.3	0.0	10.0	12	2	10
17	13	S	36.8	30.0	44.4	38	20	18
18	1	S	85.7	80.0	88.9	14	5	9
18	2	S	38.5	28.6	80.0	26	21	5
18	3	O	33.3	16.7	50.0	12	6	6
18	4	S	29.7	14.3	50.0	37	21	16
18	5	O	9.1	4.2	22.2	33	24	9
18	6	S	52.3	71.4	50.0	65	7	58
18	7	O	65.0	50.0	87.5	20	12	8
18	8	S	43.9	41.2	45.8	41	17	24
18	9	O	27.8	25.0	33.3	18	12	6
18	10	O	24.0	14.8	47.6	75	54	21
18	11	S	62.7	59.8	69.4	118	82	36
18	12	O	35.8	35.6	37.5	53	45	8
18	13	O	41.7	50.0	25.0	12	8	4
18	14	S	68.4	75.0	57.1	19	12	7
18	15	O	78.6	75.0	80.0	14	4	10
19	1	S	35.7	35.7	—	28	28	0
19	2	O	8.0	8.0	—	25	25	0
19	3	O	16.7	16.7	—	54	54	0
19	4	S	100.0	100.0	—	2	2	0
19	5	O	44.7	26.1	62.5	47	23	24
19	6	O	31.0	21.1	50.0	29	19	10
19	7	O	45.5	25.0	100.0	11	8	3
19	8	S	66.7	71.1	25.0	42	38	4
19	9	O	14.6	9.1	16.2	48	11	37
19	10	O	17.9	16.7	50.0	56	54	2
19	11	S	51.9	20.0	59.1	27	5	22
19	12	O	15.8	19.2	8.3	38	26	12
19	13	S	15.1	14.3	16.7	53	35	18
19	14	S	20.0	20.0	—	30	30	0

Meridian No.	Population No.	Habitat	% Fused			Number of five-banded		
			All	Yellow	Pink	All	Yellow	Pink
20	1	S	41.2	80.0	25.0	17	5	12
20	2	O	17.4	0.0	26.7	23	8	15
20	3	S	51.3	50.0	51.6	39	8	31
20	4	O	52.6	57.1	50.0	19	7	12
20	5	S	40.5	33.3	44.0	37	12	25
20	6	S	50.0	33.3	57.1	10	3	7
20	7	O	20.0	0.0	37.5	15	7	8
20	8	O	48.5	46.9	100.0	33	32	1
20	9	S	57.1	42.9	64.3	21	7	14
20	10	O	46.7	37.5	57.1	30	16	14
20	11	S	0.0	0.0	0.0	29	17	12
20	12	S	42.1	33.3	57.1	19	12	7
20	13	S	52.0	100.0	50.0	25	1	24
20	14	S	12.5	0.0	14.3	8	1	7
21	1	S	23.5	12.5	33.3	17	8	9
21	2	S	50.0	42.9	60.0	12	7	5
21	3	O	15.6	21.1	7.7	32	19	13
21	4	O	3.7	6.7	0.0	27	15	12
21	5	O	39.2	31.3	52.6	51	32	19
21	6	S	25.0	18.8	33.3	28	16	12
21	7	S	42.9	50.0	33.3	7	4	3
21	8	O	35.7	26.3	55.6	28	19	9
21	9	S	37.2	31.8	42.9	43	22	21
21	10	O	10.0	0.0	18.8	30	14	16
22	1	S	33.3	—	33.3	3	0	3
22	2	O	15.4	8.3	100.0	13	12	1
22	3	O	47.8	33.3	63.6	23	12	11
22	4	S	52.7	58.3	51.9	93	12	81
22	5	O	16.9	25.6	0.0	59	39	20
22	6	S	53.4	47.1	55.4	73	17	56
22	7	S	34.0	0.0	36.4	47	3	44
22	9	S	66.7	50.0	100.0	3	2	1
22	10	O	50.0	0.0	100.0	2	1	1
22	11	S	58.0	66.7	53.1	50	18	32
22	12	O	70.0	71.4	69.8	50	7	43
22	13	O	10.0	0.0	16.7	10	4	6
22	14	S	64.7	63.6	66.7	17	11	6
22	15	S	45.9	42.1	50.0	37	19	18
23	3	O	70.0	—	70.0	10	0	10
23	4	S	58.3	—	58.3	12	0	12
23	5	S	74.1	50.0	76.0	27	2	25
23	6	O	50.0	0.0	60.0	6	1	5
23	7	O	26.7	22.2	33.3	15	9	6
23	8	S	81.0	33.3	88.9	21	3	18
23	9	S	75.0	—	75.0	8	0	8



Appendix 5. Proportion of shell surface covered with bands (%B) in five-banded shells in plots 12–17. Habitat: O – open, S – shaded

Meridian No.	Population No.	Habitat	% B			N five-banded		
			All	Yellow	Pink	All	Yellow	Pink
12	1	S	51.2	51.0	51.3	61	67	36
12	2	O	44.9	43.7	46.8	20	12	8
12	4	O	33.6	33.7	32.9	49	41	8
12	5	O	53.0	50.6	58.4	16	11	5
12	6	S	50.3	51.9	48.9	36	17	19
12	7	O	44.5	41.1	48.5	15	8	7
12	9	S	54.7	55.2	54.5	15	4	11
12	10	S	49.3	49.8	48.2	31	22	9
13	2	O	38.5	38.1	38.9	10	5	5
13	3	S	45.5	48.6	46.5	5	2	3
13	5	O	52.6	50.3	54.8	35	17	18
13	6	S	56.0	52.8	58.3	48	20	28
13	7	O	44.5	43.1	44.6	38	2	36
13	8	S	52.9	54.0	48.6	15	12	3
13	9	S	51.9	47.5	54.2	23	6	15
13	10	S	42.1	46.4	40.5	18	5	13
14	1	S	44.8	43.7	45.1	43	8	35
14	2	S	46.3	49.7	44.7	16	5	11
14	3	O	42.0	40.7	42.4	35	9	26
14	5	S	41.9	42.3	40.9	19	14	5
14	6	O	41.8	–	41.8	9	0	9
14	7	O	42.8	43.3	42.6	45	14	31
14	8	S	47.7	48.0	47.1	24	15	9
14	9	O	38.9	39.1	37.8	17	15	2
15	4	O	47.0	42.8	47.9	107	69	38
15	5	S	44.6	45.1	44.1	9	5	4
15	6	S	52.7	52.7	–	34	34	0
16	2	S	47.8	47.8	–	4	4	0
16	3	S	49.6	49.2	51.4	25	20	5
16	4	S	49.3	49.2	49.7	29	22	7
16	5	O	46.1	42.0	48.8	40	16	24
17	1	O	43.4	43.2	55.9	57	56	1
17	2	O	44.1	44.8	42.7	71	48	23
17	3	S	45.7	46.1	44.6	7	5	2
17	4	S	50.0	48.5	59.1	29	25	4
17	5	S	55.3	55.4	55.2	82	45	37
17	6	O	50.0	48.2	50.9	67	22	45
17	7	S	46.5	45.2	48.5	26	16	10
17	8	O	52.2	52.2	–	34	34	0