VARIATION IN THE SHELL COLOUR AND BANDING POLYMORPHISM OF *CEPAEA NEMORALIS* (L.) IN RURAL AREAS AROUND WROCŁAW

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ABSTRACT: 105 samples of *Cepaea nemoralis* (L.) were made in rural locations around the city of Wrocław in 2008-10. Variation in the shell colour and banding polymorphism showed no relationship to habitat, nor were there any large-scale geographical patterns. In some morphs, there were strong frequency correlations between samples close to one another, but these never extended beyond 20 km, and usually involved much shorter distances. Relative to populations within the city, these populations vary more among themselves, but are individually less polymorphic. Linkage disequilibria in common between city and country suggest a common origin. These results suggest that rural populations have a more recent origin, and that they are more isolated from one another than those in the city. A process of recent passive dispersal by humans, with some local spread appears to account for the pattern of variation observed.

KEY WORDS: terrestrial snails, polymorphism, *Cepaea nemoralis*, rural populations

INTRODUCTION

There have been many studies on the striking shell polymorphism of the European land snail *Cepaea nemoralis* (Linnaeus, 1758). A great variety of patterns of variation have been discovered (JONES et al. 1977, COOK 1998), involving, in varying proportions, forms of natural selection, founder effects, genetic drift and gene flow. Patterns vary with locality, and although there are some trends visible across the whole geographical range (JONES et al. 1977, SILVERTOWN et al. 2011), there are many deviations from these broad trends, and some very local patterns may change over short distances.

While sometimes recorded from forests, and especially from their fringes, *C. nemoralis* also flourishes in more open, usually anthropogenic habitats: hedges, roadside verges, waste ground in cities, various grasslands, gardens, parks and orchards. Within its natural range, it has colonised previously inhospitable areas in towns and cities where pollution has declined, and waste ground or gardens are plentiful (CAMERON et al. 2009). Within Poland, nearly all populations of the species are in highly modified habitats. In the south-east of the country, these populations are certainly introduced (OŻGO 2005), and it is possible that this is also true for most Polish populations of the species.

CAMERON et al. (2009) compared and contrasted variation in populations within the city limits of Wrocław (Lower Silesia, Poland) and Sheffield (central England). They found that there were marked differences in the pattern of variation between the two cities, related to the timing of colonisation and the degree of interconnectedness of populations in each city. Populations in Wrocław were more alike,

were more polymorphic, and were more interconnected than those in Sheffield, where colonisation, mostly passively induced by humans, was more recent, and populations more isolated.

Within Lower Silesia, populations of *C. nemoralis* are not confined to cities and towns, but they are concentrated near villages or along roadsides and in derelict or recently cleared sites. More natural habitats, and especially forests, are occupied by the closely-related *Cepaea hortensis* (O. F. Müller, 1774), and cultivated or grazed agricultural land is unsuitable for the species. Populations are thus less connected than in the cities, and it is probable that their distribution reflects accidental dispersal by humans. This study reports on a survey of *C. nemoralis* populations in rural habitats around Wrocław, and compares the pattern of variation found with that seen in the city and elsewhere.

AREA STUDIED

Figure 1 shows the area in which samples were made, and the location of samples within it. The area is about 58 km east-west, and 70 km north-south, though there are two little sets of outliers in the north contributing to this size. The terrain is a mixture of flat ground associated with the floodplain of the river Odra and its tributaries, and areas of low hills. The area is primarily agricultural, with some managed forests, and some areas under development as industrial parks, around which there is often waste ground awaiting development. While there are few substantial towns within the area (sampled only on their fringes), many villages have extensive and ongoing residential development, creating temporary waste ground very suitable for *C. nemoralis* populations.
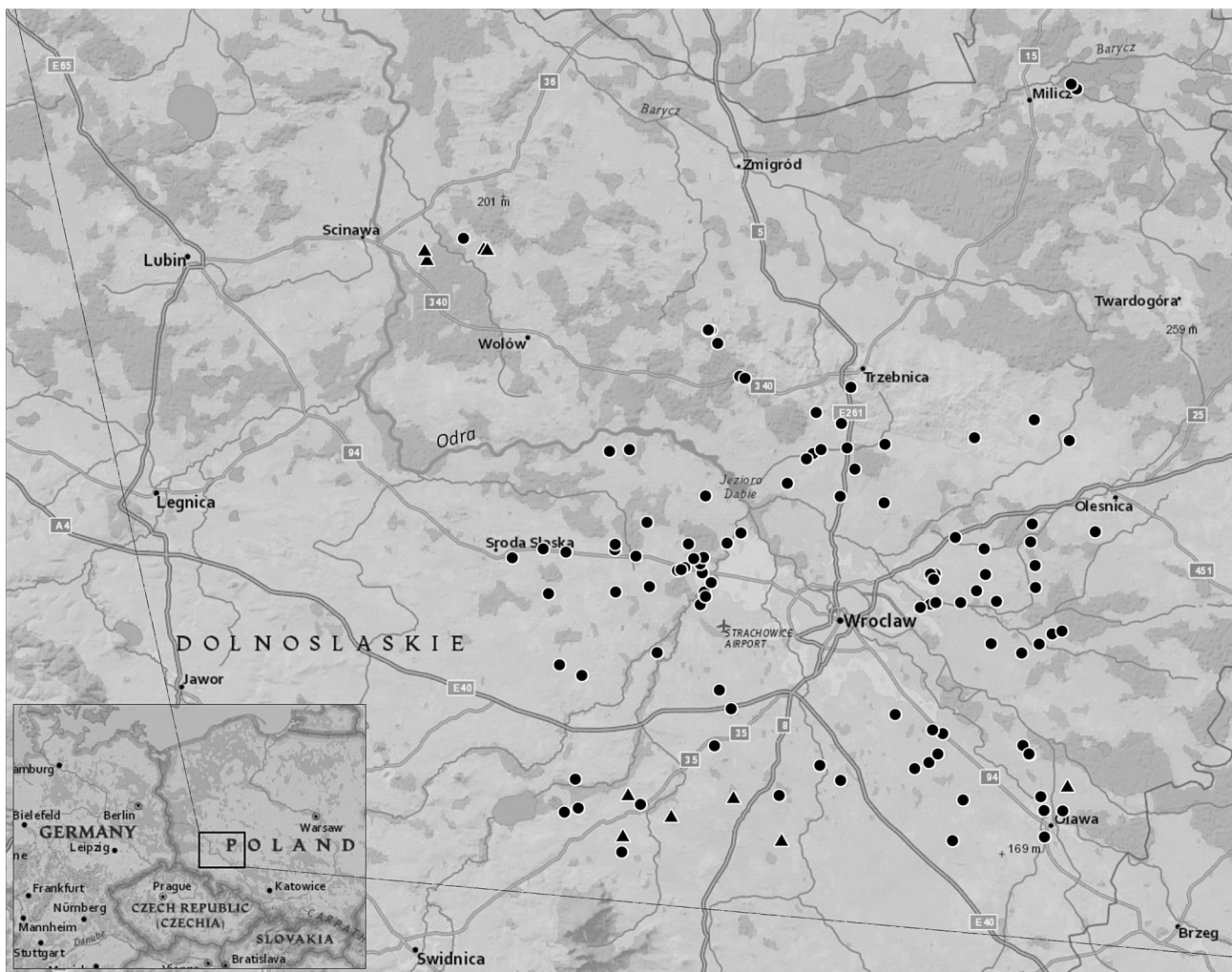

Fig. 1. The area sampled, and site locations. Black points – samples of *C. nemoralis;* triangles – samples of *C. hortensis* only. Note that the central space lacking samples is the city of Wrocław

MATERIAL AND METHODS

Journeys were made over the whole area to detect and sample *C. nemoralis* populations. In many places, no populations were found, though in some of these *C. hortensis* was present. Where populations were found, samples were made in areas of no more than 400 m², or along no more than 30 m of roadside verge. Habitats ranged from very open and grass dominated to dense but very young scrub. The majority were mixtures of grass and herbaceous vegetation with small bushes and shrubs. No populations were found in mature forest.

All adult *C. nemoralis* (live, or as fresh empty shells) were collected and scored, as were juveniles large enough to avoid confusion with *C. hortensis*. Following JONES et al. (1977), these were scored for colour of shell (yellow, pink or brown), for the presence or absence of bands, for the midbanded morph (00300) and for trifasciate (00345), with the remainder of banded shells recorded as many-banded. Live specimens were recorded in the field, and released into the sites from which they had been collected.

In all analyses, we have used morph frequencies, not estimated allele frequencies. For midbanded the frequencies are those within the banded shells; for trifasciate they are those within shells with more than one band, reflecting the dominance hierarchy at these loci (JONES et al. 1977). Where regression or least squares correlation has been used all these frequencies have been arcsine transformed. Besides tests of association of morphs with each other and with po-

sition and habitat, we have examined the linkage disequilibrium between shell colour and banding, taking simply the proportion of unbanded shells within each colour class and other associations among colour and banding classes.

Following CAMERON et al. (2009), we have used the Nei index of genetic similarity using the frequencies of yellow, unbanded, midbanded in banded and trifasciate in many-banded for a Mantel test of association with distance. Overall pattern has also been analysed by Principal Components Analysis (PCA) using the same four frequencies to assess autocorrelation among populations on the basis of overall genetic similarity. Spatial pattern has also been examined using Moran's I for each morph and for sites' scores on the first PCA axis. Variation within and among populations has been estimated via the Simpson index of diversity (SOUTHWOOD & HENDERSON 2000), by the proportions of samples with different numbers of morphs present, and by estimating F_{ST} based on morph frequencies (CAMERON et al. 2009). We estimated means and standard deviations of these F_{ST} values using a bootstrapping procedure with 1,000 permutations. The software used for the analyses comprised: SAM (RANGEL et al. 2006) for Moran's I; PC-ORD (MCCUNE & MEFFORD 1999) for Mantel test and clustering; CANOCO, version 4.5 (TER BRAAK & ŠMILAUER 2002) for PCA; and STATISTICA, version 7.1 (STATSOFT INC. 2010) for all the other one-dimensional tests.

RESULTS

We searched 114 sites at which collections were made in 2008 to 2010. 105 of these yielded samples of 10 or more usable *C. nemoralis* shells (mean sample size 88 shells), of which nine also contained *C. hortensis*. The remaining nine sites contained only the latter species (Fig. 1). Appendix 1 gives the numbers of each morph in each sample together with details of habitat (grass, intermediate or scrub) and location (village name and co-ordinates in decimal degrees). Table 1 summarises the basic data derived from these

samples. Brown shells were extremely rare, occurring at low frequency in only three samples; the great majority of non-yellow shells were pink. Although there were differences in mean frequencies of the morphs at each locus, and in the proportion of samples in which the morphs were recorded, the range of values found approaches the maximum possible (0 to 100%) in all cases.

There are no significant effects of habitat, nor are there overall trends with latitude or longitude. Within

Table 1. Mean morph frequencies overall, with the range of each, and means by habitat. No differences between habitats are significant on arcsine transformed frequencies, and some differences run contrary to those expected from other studies

	n	Yellow	Unbanded	Mid in banded	00345 in many banded
Overall mean	105	55.0	29.8	26.2	29.0
Range		4.6–100	0–91	0–100	0–100
Mean open	9	53.1	27.4	31.9	15.2
Mean intermediate	76	54.1	27.9	23.7	31.7
Mean shaded	20	59.0	38.2	32.7	24.6

Table 2. Disequilibria among banding and colour classes. Banding and colour are linked, but midbanded within banded and colour are not. *** P<0.001, * P<0.05. Null samples usually lack one of the colour or banding classes altogether; a few show exact equality in the banding class between colours

	Excess in yellow	null	Excess in pink	Chi squared
Unbanded	67	14	27	15.04***
Midbanded	31	19	55	6.70*

samples there is a trend for unbanded to be in excess in yellow rather than pink (Table 2), and, less strongly, for midbanded to be in excess in pink. There are no significant associations between morphs among samples, nor do the disequilibria we mention above show any clear geographical pattern. A Mantel test of the relationship between distance and overall genetic similarity (estimated by Nei's I) shows a slight but significant decay of similarity with distance (r=–0.14, P=0.002).

Tables 3, 4 and 5 contain comparative data for Wrocław city as well as from this study. The comparisons are discussed below. Table 3 shows the results of Moran's I tests for spatial autocorrelation at each lo-

cus, and for overall similarity using the first axis of the PCA (details of the PCA analysis are shown in Appendix 2). Few samples in the rural area were within 1 km of each other, so all pairs within 2 km have also been considered together. Even so, the numbers of samples in different distance classes vary greatly, which affects the magnitude of the index required to achieve significance. Both unbanded and midbanded in banded show significant positive associations in the smaller distance classes, as does the first axis of the PCA. Yellow and trifasciate show no such significant correlations, but in all cases the index tends to be greatest and positive at the shorter distances, and negative at the longest. An alternative analysis of the first PCA axis, creating distance classes with equal numbers of samples, indicates that there are strong positive associations up to about 20 km distance, but negative ones, or no relationship beyond that point.

Table 4 shows the values for F_{ST} at each locus, with standard deviations and confidence limits. In each case, values are higher than in the city, and extreme morph frequencies are found more often (Fig. 2). Table 5 shows three different ways of assessing within population variation. In each, the amount of such variation is less than in populations from the city.

Table 3. A – values of Moran's I for each morph, and for the scores on the first axis of a PCA for samples used in this study, and for those within the city of Wrocław (CAMERON et al. 2009). Only probabilities less than 0.05 are given; ns – not significant. Values of Moran's I are given for all pairwise comparisons less than 2 km apart, as there are few samples in the shorter distance classes used in Wrocław (see text). B – values of Moran's I for scores on the first PCA axis, using distance classes with equal numbers of paired samples in each distance class

A						
Distance classes (km)		This study			Wrocław	
Yellow	N	Index	p	n	index	p
0–0.5	15	–0.150	ns	49	0.365	0.020
0.5–1.0	16	0.255	ns	60	0.128	ns
1.0–2.0	46	0.036	ns	137	0.217	0.016
Less than 2.0	*77*	*0.045*	*ns*	*246*	*0.225*	*0.003*
2.0–4.0	138	–0.057	ns	477	–0.054	ns
4.0–8.0	332	0.075	ns	1111	0.024	ns
8.0+	4912	–0.015	ns	794	–0.117	0.006
Unbanded						
0–0.5	15	0.530	0.045	49	0.154	ns
0.5–1.0	16	0.099	ns	60	0.145	ns
1.0–2.0	46	0.407	0.006	137	0.049	ns
Less than 2.0	*77*	*0.367*	*0.006*	*246*	*0.093*	*ns*
2.0–4.0	138	0.288	0.003	477	0.011	ns
4.0–8.0	332	0.214	0.002	1111	–0.063	0.018
8.0+	4912	–0.039	0.001	794	0.007	ns

Table 3. cont.

Midbanded

0–0.5	15	0.605	0.024	49	0.520	0.002
0.5–1.0	16	0.564	0.022	60	0.506	0.002
1.0–2.0	46	0.155	ns	137	0,325	0.002
Less than 2.0	*77*	*0.327*	*0.006*	*246*	*0.408*	*0.001*
2.0–4.0	138	0.231	0.006	477	0.031	ns
4.0–8.0	332	0.192	0.002	1111	–0.103	0.004
8.0+	4912	–0.035	0.001	794	–0.051	ns

Trifasciate

0–0.5	15	0.291	ns	49	0.105	ns
0.5–1.0	16	0.343	ns	60	–0.075	ns
1.0–2.0	46	0.011	ns	137	0.084	ns
Less than 2.0	*77*	*0.131*	*ns*	*246*	*0.049*	*ns*
2.0–4.0	138	0.040	ns	477	0.041	ns
4.0–8.0	332	0.183	0.006	1111	–0.016	ns
8.0+	4912	–0.026	0.003	794	–0.063	ns

PCA 1

0–0.5	15	0.472	ns	49	0.395	0.01
0.5–1.0	16	0.641	0.017	60	0.105	ns
1.0–2.0	46	0.240	ns	137	0.335	0.004
Less than 2.0	*77*	*0.369*	*0.004*	*246*	*0.291*	*0.001*
2.0–4.0	138	0.124	ns	477	0.041	ns
4.0–8.0	332	0.224	0.001	1111	–0.047	ns
8.0+	4912	–0.035	0.001	794	–0.095	0.012

B

Distance Class	Count	Upper limit (km)	Moran's I	P
1	911	11.835	0.193	0.001
2	910	18.789	0.106	0.006
3	910	24.457	–0.122	0.002
4	910	30.997	–0.212	0.001
5	910	38.307	–0.025	ns
6	909	79.286	0.002	ns

Table 4. Estimates of F_{ST} (adjusted for sampling error) based on morph frequencies at four loci in this study, and in Wrocław (CAMERON et al. 2009). The estimates of means and standard deviations are derived from a bootstrapping procedure with 1,000 permutations

This Study	Yellow	Unbanded	Midbanded	Trifasciate
F_{ST}	0.137	0.249	0.314	0.312
estimated mean of F_{ST}	0.137	0.251	0.320	0.322
estimated SD of F_{ST}	0.021	0.028	0.038	0.032
Wrocław City	Yellow	Unbanded	Midbanded	Trifasciate
F_{ST}	0.089	0.092	0.123	0.111
estimated mean of F_{ST}	0.086	0.091	0.123	0.110
estimated SD of F_{ST}	0.019	0.015	0.021	0.018

Table 5. Within population variation in samples from this study, and in Wrocław, estimated by the proportion of samples monomorphic at each of the major loci, by the number of morphs present (ignoring the very rare brown shells in each and considering combinations of colour and each of unbanded, midbanded and many-banded), and by the values of the Simpson Index of Diversity. *One sample lacked any shells with more than one band

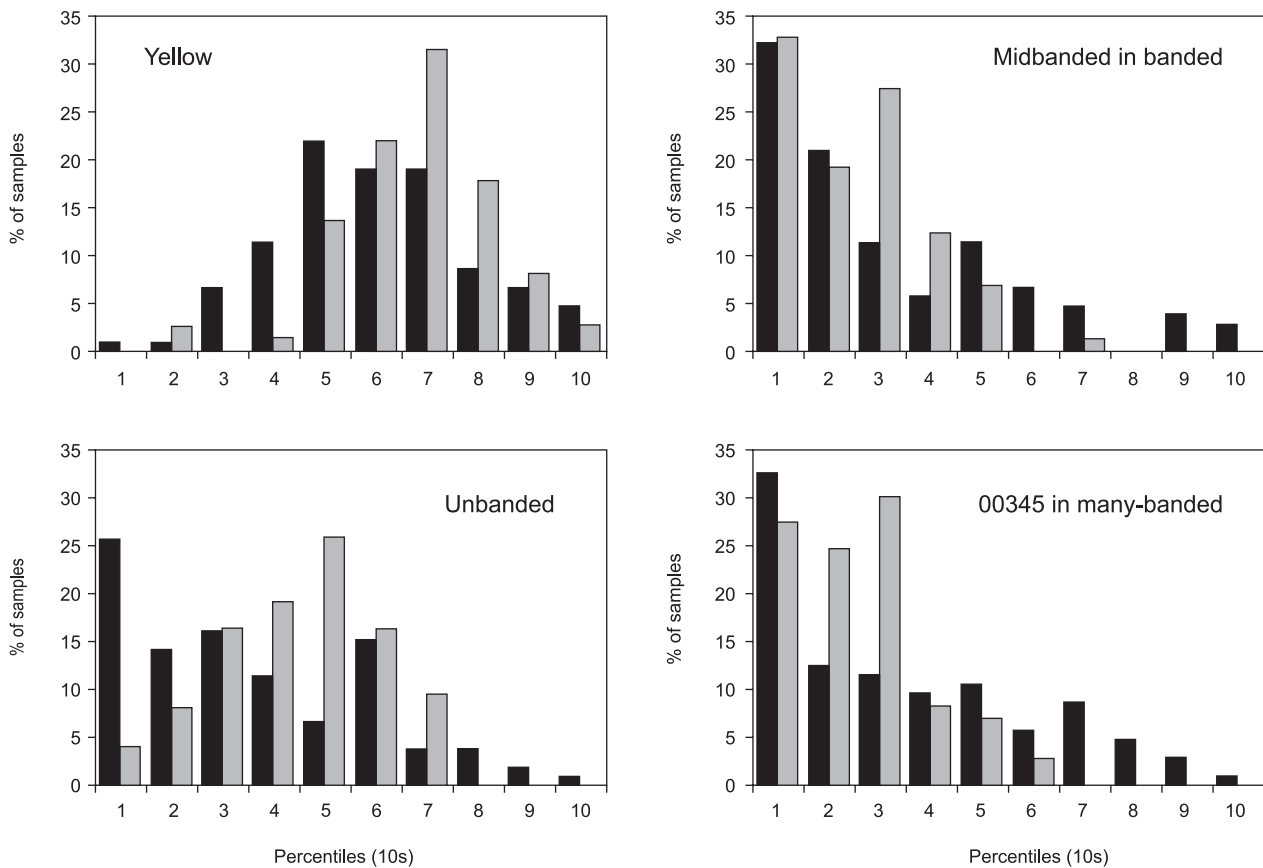|	This study		Wrocław	
	n	%	n	%
Number of samples	105		73	
Monomorphic				
For colour	1	0.9	0	0
For banding +/−	13	12.4	0	0
For midbanded +/−	18	17.1	7	9.6
For trifasciate +/−	19*	18.1*	6	8.2
Number of morphs				
1	0	0	0	0
2	5	4.8	0	0
3	5	4.8	0	0
4	24	22.9	7	9.6
5	23	21.9	12	16.4
6	48	45.7	54	74.0
Simpson Index				
Index	0.658		0.730	
variance	0.022		0.004	

Fig. 2. The frequency distribution of samples among 10% bands of morph frequencies for each of the four loci studied. Black bars, this study; grey bars, samples from Wrocław city

DISCUSSION

Variation among the samples used in this study shows no effect of habitat, nor is there any broad geographical trend. Although varying in strength among morphs there is a pattern of micro-geographical variation, with populations close to one another being similar. Such variation is widespread, and is found even when habitat effects are also evident (JONES et al. 1980, CAMERON & DILLON 1984, CAMERON & PANNETT 1985). It is also found in studies of molecular variation (ARNAUD et al. 1999, BELLIDO et al. 2002). There are no patterns remotely resembling the "Area Effects" of CAIN & CURREY (1963), where large numbers of nearby populations have near-identical and often extreme morph frequencies. These populations vary greatly, with frequencies of each morph ranging over most of the possible range.

The comparison with similar data from within Wrocław itself (CAMERON et al. 2009) assists us in interpreting this pattern. The populations in this study share with those from the city the lack of habitat associations, attributable to the very recent character of the more shaded habitats. They also share a pattern of spatial autocorrelation (Table 3), although, in detail, there are differences in the strength of this pattern in some morphs in the two studies. They differ from those in the city in the distance over which such autocorrelation is significant and positive (greater in this study), in the lower levels of within population genetic diversity (Table 5), and in the greater degree of variation in morph frequencies among sites (Table 4, Fig. 2). While there are some differences in mean morph frequencies between the two sets of samples, these are not great, and the overlap in frequency range within each is great. In comparison with another city (Sheffield, England), CAMERON et al. (2009) concluded that populations in Wrocław were dense and interconnected, and had occupied the city for many decades. While some populations in this study were dense, many occupied very small and isolated patches of suitable habitat, with no evident connection to others.

The most probable explanation of these results is that populations of *C. nemoralis* are very recently established, that they have reached their present locations as a result of accidental transport, often in small numbers, by humans, and not necessarily from nearby locations. We note that most sites occupied are disturbed and short lived within villages or industrial developments, and sites occupied previously become obliterated by development as new ones are created. Although selection regimes might differ among sites, even powerful selection of the kind reported by OŻGO (2011) is obscured by the temporary and recent character of most populations. Hence the pattern of variation is a product of multiple founder effects with some evidence of local movement. Classically, founder effects were presumed to result in loss of genetic diversity, but as OŻGO (2011) points out the species is hermaphrodite, has multiple matings and long term sperm storage. A single multiply-mated individual can carry a considerable proportion of the population's genetic variation at these loci. Its progeny will carry a biased but not drastically reduced sample of the variants present in the parental population. A very similar pattern has been reported for the region of Gdańsk (CAMERON et al. in press), where there are effects of habitat, but where human transport appears to account for the general distribution, coupled with short range dispersal from established populations.

In the case of Gdańsk, there is evidence to suggest that the present widespread distribution of *C. nemoralis* is recent. Unfortunately, we lack historic records to test this idea within this area (absence of records being no proof of actual absence). We know that *C. nemoralis* was present in the city of Wrocław before the Second World War (CAMERON et al. 2009), but not whether it was present outside. We have one piece of indirect evidence. To the south, the small town of Kudowa Zdrój now has dense and abundant populations of *C. nemoralis* (POKRYSZKO & CAMERON unpublished data). The mollusc fauna of the area was surveyed in detail by A. WIKTOR in the 1960s, and he reported no *C. nemoralis* (WIKTOR 1964). Given the conspicuousness of the snail and the competence of the recorder, this is good evidence for real absence. Further south still, the evidence from the Czech Republic indicates that *C. nemoralis* has spread recently and rapidly, and is strongly associated with human activities (HONĚK 1995, DVOŘÁK & HONĚK 2004). It seems likely that Silesia as a whole has been colonised relatively recently, and that loci of spread formed initially in cities.

There is one peculiarity of populations from both the city of Wrocław and the surrounding countryside. Populations from northern Poland generally show linkage disequilibrium between colour and banding such that unbanded is more frequent in pink than in yellow shells (WAGNER 1990, CAMERON et al. 2011). WAGNER (1990) found that this disequilibrium disappeared further south or was even reversed, as it is both in the city and the countryside in this study, and in some populations near Wałbrzych (WAGNER 1990, POKRYSZKO unpublished data). The reasons for these disequilibria persisting are unknown, but the consistency of the pattern suggests a recent common origin of populations in the region.

COOK (1998) made a powerful case that aspects of this variation in *C. nemoralis* were a product of migra-

tion and gene flow, and in particular that the lepto-kurtic transport of individuals (some travelling much greater distances than possible by active dispersal) helped to maintain the polymorphism, which is found in nearly all populations. Our results and those of similar studies referred to earlier suggest that newly occupied territory such long-distance dispersal sets up founder populations, the composition of which influences the genetic constitution of other nearby populations derived from them. This hypothesis is testable by use of molecular markers.

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Appendix 1. Details of samples made. Longitude and Latitude are shown in decimal degrees east and north. Place name indicates the nearest village. Habitat: 2 – scrub, shaded; 3 – intermediate; 4 – grass dominated and open. Y – yellow; P – pink; B – brown; 0 – unbanded; 3 – midbanded, 00300, 00345, trifasciate; 5 – more than three bands, typically 12345

Date	Long	Lat	Place name	Serial no.	Habitat	Cn tot	Y0	Y3	Y00345	Y5	P0	P3	P00345	P5	B0	B3	B5
2008	17.36078	51.17815	Bystre	1	3	120	31	35	30	5	3	12	1	3			
2010	17.0351	50.97855	Żórawina	2	2	110	55	3	21	3	9	1	14	4			
2010	16.8263	51.1472	Żar 1	3	3	109		13	28	37		5	9	17			
2010	16.66178	51.12867	Kryniczno	4	3	120			89	15			12	4			
2010	16.69577	50.97958	Milin	5	3	103	40	3	2		24	12	16	6			
2010	16.68385	51.16173	Juszczyn	6	3	158	50		43	15	9		28	13			
2010	16.61558	51.15725	Środa Śląska	7	3	108		1	70	34			2	1			
2010	17.18147	51.17355	Długołęka	8	3	153	23	15	35	12	6	15	28	19			
2010	17.28277	51.26778	Siekierowice	9	2	25	5	4	1	2	1	6	5	1			
2010	16.65477	51.16433	Komorniki	10	3	84			4	6			52	22			
2010	16.74633	51.16387	Błonie 1	11	3	230			139	88			1	2			
2010	16.8695	51.1374	Leśnica1	12	3	74	4	6	12	25	3	6		29			
2010	17.04787	51.2936	Trzebnica	13	3	88		11	12	12		12	26	15			
2010	17.29073	50.96523	Osiek	14	3	171	25	14	18	28	10	21	36	19			
2008	17.1782	50.93002	Wierzbno	15	3	133	22	9	10	13	11	21	21	14	6	5	1
2008	16.7567	50.9341	Rogów Sobócki 2	16	3	10	4	1		1	4						
2008	17.2843	51.1334	Jenkowice	17	3	80	8	24	36	0	3	6	3	0			
2010	16.70407	51.06295	Wilków Średzki	18	2	137	11	3	6	23	38	20	24	12			
2010	17.0533	51.2283	Ligota Piękna 1	19	3	40		19	6	3	1	8	1	2			
2010	17.319	50.9538	Oława 2	20	3	48	8	3		15		8		14			
2010	17.00848	50.99051	Zórawina	21	3	169	57		26	25	18		16	27			
2009	17.289	51.088	Chrząstawa Mała	22	3	138	104	4		13	11	3	2	1			
2010	16.90775	51.17727	Pracze Odrzańskie	23	3	117	22	12	2	24	10	23		24			
2009	17.21865	51.16452	Kamień	24	3	81	8	5	2	9	16	22	10	9			
2009	17.3272	51.2511	Dobroszyce	25	3	40	11	1	3	2	3	3	15	2			
2009	17.2202	51.14387	Piecowice	26	3	122	27	1	3	8	84	2	5	4			
2008	17.1481	50.9928	Sulęcin 1	27	3	37	5	3	6	7	2		10	2			
2008	17.19168	50.96277	Sobocisko	28	3	38	14	1	1	5	5		6	6			
2008	17.278	51.1698	Ręków	29	2	117	73	1	20	8	8		4	3			
2008	17.209	51.1307	Śliwice	30	3	57	28		4	4	14		5	2			
2008	17.15565	51.1443	Kolonia Kiełczów 2	31	3	180	52		7	23	56	4	17	21			
2008	16.8311	51.1478	Żar 2	32	3	28	3		9	7	1		4	5			
2008	17.13695	51.11742	Swojczyce	33	2	56	15	1	7	14		9	2	8			

Date	Long	Lat	Place name	Serial no.	Habitat	Cn tot	Y0	Y3	Y00345	Y5	P0	P3	P00345	P5	B0	B3	B5
2008	16.80068	51.08107	Romnów	34	3	44	6	4	6	4	4	12		8			
2008	16.95673	50.9663	Pełczyce	35	3	35	3		2	4	2		8	16			
2010	16.9916	51.2363	Strzeszów 3	36	3	105		37	2	5		51	6	6			
2008	17.2341	51.1222	Pietrzykowice	37	3	106	61			10	21	5		9			
2008	17.0436	51.2451	Wisznia Mała	38	3	135	1	30	16	43	19	11	15	0			
2008	16.68177	50.95298	Proszkowice	39	3	21	2			3		2	5	9			
2008	17.2273	51.0884	Nadolice Małe	40	3	56	2	2	15	11	1	6	9	10			
2010	16.74718	51.16797	Błonie 3	41	3	109	5		26	9	11		48	10			
2008	16.7909	51.134	Lutynia	42	3	53	25	1	2	6	5	3	5	8			
2010	17.1045	51.03155	Zacharzyce	43	3	112	54	2	5	15	11	3	7	15			
2010	16.8361	51.1494	Żar 3	44	3	53		1	9	18	2	5	9	9			
2010	17.2663	51.081	Nadolice Wielkie	45	3	65		3	15	34		1	7	5			
2008	16.9668	51.217	Szewce	46	3	164		27	11	17	5	73	10	21			
2010	16.5532	51.4125	Konary 1	47	4	12		5	1	2	4		0	0			
2008	16.76508	51.24397	Księginice	48	3	195	16	12	24	51	41	5	6	40			
2010	17.3053	51.0962	Chrząstawa Wielka 1	49	4	145	19		25	48	39		3	14			
2008	17.1658	51.01607	Groblice	50	3	80	27	1	2	4	17	14	3	12			
2008	16.9997	51.2408	Strzeszów 2	51	3	205		38	10	29	63	39	10	16			
2008	16.8694	51.3393	Osola 2	52	4	176		82	7	13	26	44		4			
2008	16.77941	50.959	Mirosławice	53	2	111	46		4	33	15	1	7	5			
2010	17.3297	51.53545	Ruda Milicka 1	54	2	22		1			13	7	1	1			
2008	17.1542	51.1398	Kolonia Kiełczów 3	55	3	82	29	2	5	13	12	4	2	15			
2008	17.1887	51.1214	Kiełczówek	56	3	87	33	1	4	16	13	2	4	14			
2010	17.15013	51.11995	Wilczyce	57	4	132	34	2	3	15	27	11	6	34			
2008	16.7555	50.9207	Rogów Sobócki 1	58	4	87			4	25	9		14	35			
2008	16.89528	51.03603	Biskupice Podgórne	59	3	24	9	2		5	4	3	1				
2010	17.2062	51.2534	Zaprężyn	60	3	94	24	7	4	20	10	22	2	5			
2008	17.1502	51.1442	Kolonia Kiełczów 1	61	3	97	40		3	14	18	2	3	17			
2008	16.8654	51.339	Osola 3	62	3	36	5	2	2	8	4	4	2	9			
2008	17.1566	51.1214	Wilczyce	63	2	30	11	2		6	4	4		3			
2010	16.8408	51.1684	Leśnica 10	64	3	64		13	5	6		23		17			
2008	16.8555	51.1522	Leśnica 6	65	3	100		12	5	35		28	4	21			
2010	16.9061	51.3025	Oborniki Śląskie 2	66	3	43	4	2	3	15	17			2			
2008	16.89	51.16912	Marszowice	67	3	65	17	1	4	11	6	1	1	24			
2010	16.88023	51.05098	Pietrzykowice	68	2	138	41	2	15	27	27		14	11	1		
2010	16.74752	51.12972	Radakowice	69	3	198	6	2	3	29	10	19	14	115			

Date	Long	Lat	Place name	Serial no.	Habitat	Cn tot	Y0	Y3	Y00345	Y5	P0	P3	P00345	P5	B0	B3	B5
2010	16.86262	51.20678	Pisarzowice	70	3	64	2	6	1	12	3	3	4	33			
2010	16.8555	51.1198	Leśnica 4	71	2	123	58	6	3	28	17	3	1	7			
2010	17.2952	50.9541	Oława 1	72	3	56	6	2		22	3	3	1	19			
2008	17.2682	51.0065	Siedlce 2	73	2	63	7	5	4	45				3			
2008	17.0102	51.2439	Strzeszów 1	74	3	51		15			4	29		3			
2010	16.7395	51.24245	Gąsiorów	75	3	134	41	1	1	14	55	7	1	14			
2010	17.15283	51.01911	Siechnice	76	2	74	9	2	18	22	5	4	14	11			
2010	16.69988	50.9561	Maniów Wielki	77	2	70	15	6		27	2	10	1	9			
2008	16.8548	51.1554	Leśnica 8	78	3	101	13	16	1	25	9	19	1	17			
2010	17.0908	51.2012	Pasikurowice	79	3	76	3	19		9	21	19		13			
2008	17.0359	51.2648	Piotrkowiczki	80	3	60		23		19	3	10	1	4			
2008	17.2798	51.1843	Borowa Oleśnicka	81	3	117	28		21	15	11	1	26	15			
2010	17.0344	51.2064	Kryniczno 1	82	2	67	9	25		3	18	4					
2010	16.74717	51.16812	Błonie 2	83	4	139	10	9	1	46	2	27	2	42			
2008	17.0919	51.2481	Pierwoszów	84	3	70	39		1	10	12			8			
2008	16.8741	51.00633	Małuszów	85	3	41			2	21			1	17			
2008	16.78757	51.18575	Mrozów	86	2	74		4	1	31			3	35			
2008	16.8611	51.1297	Leśnica 3	87	4	186	12	11		57	18	10	2	76			
2008	16.866	51.3395	Osola 1	88	2	14	5	3			3	3					
2008	16.8625	51.1261	Leśnica 2	89	3	54		21		9		22		2			
2008	17.2838	51.151	Oleśniczka	90	4	11	6	2		2		1					
2008	17.0041	51.2734	Mienice	91	2	90		19		20	9	22		20			
2010	16.8597	51.1576	Leśnica 9	92	3	51	1	4	1	7	1	18		18			
2008	17.33662	51.53137	Ruda Milicka 2	93	2	67	7	11	1	12	12	10		14			
2008	16.77365	51.15857	Wróblowice	94	2	98	39	8		13	17	2		16		3	
2008	16.8471	51.1567	Leśnica 7	95	3	24	4			5	2	5		8			
2010	16.8779	51.3287	Morzęcin Wielki	96	4	44	1	4		11	10	3		16			
2008	16.9127	51.3008	Oborniki Śląskie 1	97	2	23	1	2	3	4	5	1	1	6			
2010	17.1295	50.9878	Bratowice	98	3	48		3	6	7	1		25	6			
2008	16.67567	51.07123	Świdnica Polska	99	3	185	33	3		46	35	13		55			
2008	17.3179	51.0984	Chrząstawa Wielka 2	100	3	129	21	7		88		5		8			
2008	16.8583	51.145	Leśnica 5	101	3	106	25	3	18	25	12	4	11	8			
2008	17.2756	50.9999	Siedlce 3	102	3	15	5			1	1			8			
2010	17.2766	50.999	Siedlce 1	103	3	79	17			14	16			32			
2010	17.1593	50.9997	Grodziszów	104	3	30	11		4	15							
2010	17.29552	50.9329	Marszowice 2	105	3	102				80				22			

Appendix 2. Site scores and Eigenvalues for the four axes of the Principal Components Analysis of morph frequencies of yellow, unbanded, midbanded in banded and trifasciate in many banded

Eigenvalues	0.345	0.278	0.200	0.177					
Loc. No.	PCA1	PCA2	PCA3	PCA4	Loc. No.	PCA1	PCA2	PCA3	PCA4
2	−1.9533	0.1986	0.1315	1.7023	54	2.8955	0.8677	−0.6822	2.0889
3	−0.6568	−0.9966	0.7648	−0.8990	55	−0.3198	0.8896	−0.3651	−0.0785
4	−2.1290	−1.6006	1.0146	−0.1701	56	−0.5633	0.9924	−0.5079	−0.1669
5	−0.4419	0.0891	−0.3674	2.7271	57	0.1926	1.0362	0.5108	0.2490
6	−1.6617	−0.3727	−0.1828	0.7362	58	0.2066	−0.8530	−1.4725	−0.8319
7	−2.0828	−1.1500	1.4512	−0.8645	59	0.1789	1.2024	0.7671	0.4457
8	−0.6573	−1.0558	0.1648	0.8556	60	0.4962	0.4456	0.7046	0.2737
9	0.0985	−0.9635	0.6148	1.7064	61	−0.4805	1.2659	−0.7948	−0.1248
10	0.0479	−2.2822	−2.1743	0.6399	62	0.3064	−0.0898	−0.4086	−0.3656
11	−2.0383	−0.9900	1.4864	−1.1114	63	0.5954	0.8789	−1.0546	−0.3777
12	0.6387	−0.3129	−0.4492	−1.3607	64	1.4131	−1.0098	0.4314	−0.1575
13	0.0316	−1.7417	−0.2597	0.4289	65	0.9082	−0.8199	0.3532	−0.7938
14	−0.2529	−0.8347	−0.0817	0.6126	66	−0.2428	0.8858	−0.6898	−0.3080
15	0.1644	−0.6535	−0.2274	1.2453	67	−0.0881	0.4123	−0.8892	−0.7310
16	0.5849	2.2825	0.3066	0.8098	68	−1.0110	0.4807	−0.5786	0.3639
17	−1.5377	−1.4252	2.0892	1.6145	69	1.0034	−0.868	−1.6940	−0.7989
18	0.2563	−0.4359	−1.0214	1.1195	70	0.7752	−0.6953	−1.0530	−0.9734
19	0.1534	−1.2226	2.2355	0.8447	71	−0.5654	1.6707	0.3534	−0.3279
20	0.6168	0.0770	0.0863	−1.1852	72	0.2451	−0.0054	−0.4156	−1.4690
21	−1.1219	0.2967	−0.5021	0.1601	73	−0.9422	0.3102	1.4183	−2.2535
22	−0.7483	2.5231	0.8978	0.3725	74	2.6539	−0.5055	1.0511	0.4196
23	0.8437	0.3306	0.2548	−0.4479	75	0.3551	1.6288	−1.1645	0.5111
24	0.8798	−0.5377	−0.428	1.2472	76	−1.0103	−0.5630	0.2356	−0.2677
25	−0.8568	−0.9718	−0.7814	1.7785	77	0.2223	0.4765	0.6957	−1.1097
26	−0.4001	1.5121	−2.2189	1.8915	78	0.8502	0.1794	0.5398	−0.5662
27	−0.7907	−0.9906	−0.0084	0.5818	79	1.5141	0.3386	0.3793	0.3035
28	−0.7275	0.4778	−0.7863	0.3918	80	0.8412	−0.1562	1.7673	−1.0064
29	−2.1986	1.0025	0.3439	1.1768	81	−1.0861	−0.4933	−0.7008	0.5760
30	−1.5304	0.9841	−0.8149	1.4022	82	1.7650	0.9209	1.7058	0.7252
31	−0.4713	0.7473	−1.3016	0.7194	83	0.7895	−0.3402	−0.0783	−1.1512
32	−1.0911	−0.8358	−0.149	−0.3943	84	−0.7872	2.0682	−0.5717	−0.4644
33	−0.3038	0.0411	0.4507	−0.3059	85	−0.0344	−0.6049	−0.4350	−1.9980
34	0.6302	−0.4342	0.2901	0.5704	86	0.2918	−0.6719	−0.5959	−1.7998
35	0.1985	−1.0112	−1.8144	−0.2749	87	0.5835	−0.1267	−0.7817	−1.2453
36	1.5014	−1.4016	1.3854	1.1254	88	1.8174	1.5147	1.8781	1.3201
37	−0.1788	2.2687	−0.2152	0.0049	89	1.7603	−0.4258	1.8430	−0.5401
38	−0.2417	−0.5779	0.9567	0.0095	90	0.1579	1.8541	2.1715	−0.1948
39	0.5582	−1.1169	−1.5473	−0.2641	91	1.4035	−0.2753	0.3701	−0.6769
40	−0.4838	−1.2916	−0.0656	−0.0729	92	1.6383	−0.8563	−0.4674	−0.4008
41	−0.8781	−1.6964	−1.2589	0.9689	93	1.0221	0.3041	0.1065	−0.2866
42	−0.5454	0.8762	−0.3018	0.5510	94	0.2869	1.5240	0.0511	−0.1992
43	−0.7983	1.1068	−0.2072	0.1636	95	0.9942	0.1319	−0.6975	−0.6636
44	−0.2949	−1.1127	−0.2042	−0.5830	96	0.9436	0.0057	−1.0251	−0.8109
45	−1.0033	−0.8063	0.8111	−1.4108	97	0.0789	−0.2149	−0.6200	−0.0759
46	1.3265	−1.2812	0.4611	0.6812	98	0.0430	−2.0536	−1.2431	1.0294
47	0.3589	0.2139	1.5182	0.7829	99	0.4663	0.6062	−0.9437	−0.7798
48	−0.1615	0.0147	−0.4925	−0.3874	100	−0.6165	0.5323	1.1236	−2.1850
49	−0.8517	0.3927	−0.5029	−0.3647	101	−0.9729	−0.0096	0.0269	0.1380
50	0.5784	0.7733	−0.3555	1.0462	102	0.2739	0.6474	−1.5566	−0.9044
51	0.9762	−0.2214	0.0738	0.9897	103	0.2831	0.6964	−1.6076	−0.8451
52	1.1969	−0.4316	1.9630	0.7839	104	−1.5758	0.9551	1.0529	−1.4231
53	−1.0275	1.2030	−0.1454	−0.4112	105	−0.4680	−0.1719	0.4914	−2.6286