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# Inferring microevolution from museum collections and resampling: lessons learned from *Cepaea*

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Studies documenting Human-Induced Rapid Evolutionary Change (HIREC) routinely compare contemporary allele or morph frequency distributions with historical baselines. All too often, this involves the re-sampling of a population that was sampled at a single time point in the past. However, year-to-year fluctuations in magnitude and direction of evolutionary response may make such studies prone to erroneous conclusions, where long-term evolutionary trends are inferred from what in fact are short-term fluctuations. Here, we explore this problem by re-sampling three Dutch populations of the land snail *Cepaea nemoralis*, whose shell colour polymorphism is known to be under thermal and predatory selection. Each of these three populations was originally sampled in at least two different years in the past. We show that conclusions on evolutionary change are strongly dependent on which of the historical sample dates is used for comparison with the contemporary sample. Our study highlights the fact that year-to-year variation in allele frequencies may often be so strong that a simple two-point comparison is unreliable to detect long-term evolutionary trends.

1 Inferring microevolution from museum collections and resampling: lessons  
2 learned from *Cepaea*

3

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13

14

## 15 **Abstract**

16

17 Studies documenting Human-Induced Rapid Evolutionary Change (HIREC) routinely  
18 compare contemporary allele or morph frequency distributions with historical baselines.  
19 All too often, this involves the re-sampling of a population that was sampled at a single  
20 time point in the past. However, year-to-year fluctuations in magnitude and direction of  
21 evolutionary response may make such studies prone to erroneous conclusions, where  
22 long-term evolutionary trends are inferred from what in fact are short-term fluctuations.  
23 Here, we explore this problem by re-sampling three Dutch populations of the land snail  
24 *Cepaea nemoralis*, whose shell colour polymorphism is known to be under thermal and  
25 predatory selection. Each of these three populations was originally sampled in at least  
26 two different years in the past. We show that conclusions on evolutionary change are  
27 strongly dependent on which of the historical sample dates is used for comparison with  
28 the contemporary sample. Our study highlights the fact that year-to-year variation in  
29 allele frequencies may often be so strong that a simple two-point comparison is  
30 unreliable to detect long-term evolutionary trends.

31

32

33 **Introduction**

34

35 As humans rapidly alter environmental conditions, opportunities for natural populations  
36 to evolutionarily adapt to such changes are increasing (Schilthuizen & Kellermann,  
37 2013). Several examples of such rapid, contemporary evolution are already available  
38 (e.g., Cheptou et al., 2008; Buswell et al., 2011). In many cases, evidence comes from  
39 the re-sampling of populations that have been sampled before, with historical data  
40 coming either from the literature or from natural history collections (Suarez & Tsutsui,  
41 2004; Schilthuizen et al., 2015). One frequently under-appreciated factor in such  
42 analyses is the temporal scale, which is important for three reasons. First, the time  
43 scale at which populations respond adaptively, depends on the organism's generation  
44 time and generation overlap. Second, the time scale at which environmental changes  
45 take place may differ: gradual long-term trends may mask many short-term, more erratic  
46 changes (Siepielski et al., 2009). Third, sampling and re-sampling itself has a temporal  
47 scale that may or may not match the scale at which environmental changes and  
48 adaptive responses take place: it may consist of just a few time points, several, or even  
49 hundreds, of generations apart, or it may consist of an uninterrupted time series.

50

51 One system frequently employed for studies of contemporary adaptive evolution is shell  
52 colour evolution (often driven by thermal selection) in the land snails *Cepaea nemoralis*  
53 and *C. hortensis*. Shell colour in these species derives from well-studied genetic  
54 polymorphisms; the shells can be yellow, pink, or brown, and bear up to five dark brown  
55 spiral bands; effectively unbanded (eU) are shells with at least the two upper bands  
56 missing. Selection by climate and visually hunting predators can result in shifts in the  
57 frequencies of colour and banding morphs (reviews in, e.g., Cook 1998, Özgo 2008).  
58 The animals are relatively sessile and easily sampled, and the species have been the  
59 subject of more than a century of investigations into local adaptation. For these reasons,  
60 several studies in *Cepaea* have made use of re-sampling to investigate temporal  
61 changes in shell colour frequencies in response to environmental change. These range  
62 from studies encompassing several years (e.g. Goodhart 1973, Arthur, Philips and

63 Mitchell 1993) to those spanning decades (e.g. Cook, Cowie and Jones 1999, Cameron  
64 2001, Cameron and Pokryszko 2008, Cameron, Cook and Greenwood 2013). Most are  
65 geographically localized, but some encompass large areas. One of the largest citizen-  
66 science projects covered the whole of Europe (Silvertown et al. 2011).

67

68 In this paper, we further illustrate the problems that may ensue when assessing the  
69 degree of evolutionary change by insufficient re-sampling of a single population. We  
70 identified several large samples of *Cepaea nemoralis* suitable for this purpose in the  
71 collection of Naturalis Biodiversity Center in Leiden, the Netherlands. Then, we  
72 resampled these populations in 2010. We examined the frequency changes in three  
73 aspects of shell morph variation, namely, (1) shell ground colour; (2) shell spiral  
74 banding; and (3) relative frequency of yellow, effectively unbanded shells. (Details of  
75 scoring schemes are explained in the Materials and Methods). We show that the  
76 conclusions depend very strongly on the year with which our recent samples are  
77 compared. This suggests that, as shown in time series in a related species, *Theba*  
78 *pisana* (Johnson 2011), year-to-year evolutionary changes are often so large that they  
79 may mask, and lead to misleading interpretations of, long-term evolutionary trends.

80

81

## 82 **Materials and Methods**

83

### 84 **Historical Baseline Data**

85 We used the dry Mollusca collection of Naturalis Biodiversity Center to locate large  
86 samples of *Cepaea nemoralis* from the Netherlands with locality information precise  
87 enough to make re-sampling possible. We identified three such sets of samples (at the  
88 time of writing, these samples had not yet received collection numbers; however, they  
89 were held in the special “large *Cepaea* sample” section):

90 (1) Lobith. Shells collected by H. Wolda and students on 20th April 1960 (N=368), 27-  
91 28th April 1961 (N=398), and 10-14th April 1962 (N=1657) at 13 positions along a 400-  
92 m section of dike on the river bank along the Rhine (51.859° N 6.085° E). The samples  
93 were used for a study on stability of a steep c. 20-m-wide cline located along the

94 section, where unbanded yellow shells increase in frequency at the expense of banded  
95 yellow and unbanded “red” shells (Wolda, 1969). Wolda (1969) reports on a total of  
96 26,230 individuals, and writes that most samples consisted of mostly living snails and a  
97 small proportion of dead shells. Since Wolda states that all living snails were returned to  
98 their collection localities after scoring, we conclude that the Naturalis samples (N=2423)  
99 consist only of snails that were collected as empty shells. Wolda (1969) also reports that  
100 the vegetation along the dike grades from a dense river-dune vegetation on sand, rich in  
101 nutrition and calcium, to more open river-dune vegetation on coarse, rich, and dry sand  
102 with calcium.

103 (2) Empe. Shells collected by J.C. van Heurn in May 1915 (N=468) and July 1951  
104 (N=340) at Empe estate (52.145° N 6.142° E). The sample of 1915 is labelled as  
105 follows: “Collected at random in one site on the main road from Zutphen to Voorst, in  
106 the Zutphen municipality, directly opposite the manor “Empe”. The collection consists of  
107 451 adults and 17 juveniles. [...] The habitats are the elm stems as well as the roadside-  
108 verges. In the verges there were no nettles or generally tall herbs, but there was a row  
109 of elm-bushes, mixed with some hawthorn, grasses and other low herbs [translated  
110 from Dutch by M.S.]”. The sample of 1951 is labelled, “Road south of Voorst. Opposite  
111 “Empe””. We conclude that both samples were taken at the exact same locality.

112 (3) Allemansgeest. Shells collected by W.C. van Heurn in summer 1942 (N=500) and  
113 1943 (N=772) on the 150 x 50 m peninsula “Allemansgeest” at the confluence of Vliet  
114 and Korte Vliet, near Voorschoten (52.143° N 4.468° E). The location and samples were  
115 reported on by Van Heurn (1943, 1945). He describes the habitat as “a narrow spit of  
116 land, planted with willow, ash, and some alder, among which a wild herb vegetation  
117 appears in summer, consisting mostly of nettles [translated from Dutch by M.S.]”. He  
118 also reports on a set of samples from 1941, which we could not locate. The collected  
119 numbers reported for 1942 and 1943 were, respectively, 535 and 787, suggesting that a  
120 small number of individuals had been lost from the samples. We assume these to have  
121 been random subsets.

122 (A fourth set, from the vicinity of Eenrum, was already reported on previously; Özgo &  
123 Schilthuizen, 2012.)

124

## 125 **Resampling in 2010**

126 We visited these exact locations in 2010. All sites were identifiable and still contained  
127 *Cepaea nemoralis*. At Lobith, the vegetation remained as described by Wolda (1969).  
128 Since snail densities were apparently much lower than in Wolda's time, we did not  
129 sample Wolda's individual sections, but instead pooled all material from the entire 400-  
130 m section of dike (20-22 June 2010). At Empe, no patch of vegetation exactly matched  
131 the description by Van Heurn, so we sampled from two different patches facing the  
132 façade of the manor house: Empe-1 (52.1457° N; 6.1435° E; reeds, nettles; 20-22 June  
133 2010) and Empe-2 (52.1456° N; 6.1426° E; maple forest; 20-22 June 2010). At  
134 Allemansgeest, finally, the vegetation had become more park-like, with lawns, tall  
135 poplars, and only a few patches where a herbaceous layer was present. *Cepaea*  
136 densities were low, so we sampled multiple times, on 21 June, 22 August, and 13  
137 September 2010. At all sites, we collected juveniles and adults, (fresh) dead and alive.  
138 Juveniles too small to judge the colour morph accurately were not included.

139

## 140 **Shell Morph Scoring Scheme**

141 Each individual from the museum samples and the recent samples was scored following  
142 the same scoring scheme as described in Cain and Sheppard (1954). We investigated  
143 three types of changes in shell morph frequencies between the historical baseline data  
144 and the resampling data: (1) changes in shell ground colour: yellow (Y), pink (P), and  
145 brown (B); (2) changes in banding categories: mid-banded (00300), three-banded  
146 (00345), five-banded (12345), and other banding; and (3) changes in yellow, effectively  
147 unbanded (YeU).

148

## 149 **Data analysis**

150 At each site, and for each pair of samples, we performed separate chi-square  
151 goodness-of-fit tests for each of the three types of morph change (banding, colour, and  
152 proportion of YeU) to examine whether there is evidence of a change in frequency  
153 between years (Supplementary Data 5). Although we recorded the frequency of all  
154 morph categories, we excluded categories from the goodness-of-fit tests when the  
155 morph frequency in the baseline year was equal to zero. The significance level of the

156 chi-square test was set at  $p = 0.05$ , and we tested for the following changes in  
157 frequency between the baseline and resampling years. Colour: yellow, pink, and brown;  
158 shell banding: mid-banded, three-banded and five-banded; YeU: Yellow-effectively  
159 unbanded. All the analyses were done in R v. 3.2.2, and the R script is provided in  
160 Supplementary Data 1.

161

162

## 163 Results

164

165 Full results are given in Figure 1 and Supplementary Data 2-4. We found no significant  
166 differences in colour ( $\chi^2 = 2.38$ , d.f. = 2,  $p = 0.30$ ) or banding morph frequencies ( $\chi^2 =$   
167 2.03, d.f. = 2,  $p = 0.36$ ) between the two patches sampled at Empe in 2010, so we  
168 pooled the data. Altogether, our 2010 sample sizes for Lobith, Empe, and  
169 Allemansgeest were  $N = 131$ ,  $N = 189$ , and  $N = 77$ , respectively. Parts of these samples  
170 were stored as vouchers in the alcohol collection of Naturalis Biodiversity Center, under  
171 collection numbers RMNH.5004222 (Allemansgeest), RMNH.5004223-5004224 (Lobith)  
172 and RMNH.5004225-5004227 (Empe).

173

174 In Lobith 2010, there was a very high frequency of usually rare “other banding” snails,  
175 which constituted 26% of all shells and consisted mostly (12 of 13) of the 00045 form.  
176 Among the 1960, 1961, and 1962 samples there were statistically significant differences  
177 in colour, banding morph frequencies, and frequencies in yellow, effectively unbanded  
178 between consecutive years (Figure 1). For example, yellow effectively unbanded  
179 showed a drop in frequency from 54% in 1960 to 48% in 1961, followed by a significant  
180 increase to 61% in 1962. Consequently, the result of comparison with 2010 depends on  
181 which of the historic samples is considered. 1960 and 1961 give a highly significant  
182 difference in the YeU frequency, while 1962 does not. Similarly, 2010 does not show  
183 significantly different colour morphs compared with 1960 or 1961, but it does in  
184 comparison with 1962, mainly due to the proportion of brown shells.

185



186 In Empe, apparently no changes in any morph category occurred between 1915 and  
187 2010. Strikingly, however, several colour morph frequencies in the 1951 sample differ  
188 significantly from both 1915 and 2010 (Figure 1). For example, the frequency of yellow  
189 decreased from 75% in 1915 to 66% in 1951, and increased again to 77% in 2010.

190

191 In Allemansgeest (Figure 1) there are no differences in the frequency of colour,  
192 banding, or yellow effectively unbanded morphs between the 1943 and 2010 samples.  
193 Between 1942 and 2010, however, significant differences in the frequency of banding  
194 and yellow effectively unbanded are observed. This reflects the change in morph  
195 composition that occurred between 1942 and 1943: differences in the frequency of  
196 banding and yellow effectively unbanded between these samples are significant and  
197 reflect an increase in mid-banded and a decrease in five-banded.

198

199

## 200 **Discussion**

201

202 Increasing evidence shows that adaptive response of populations to short term  
203 environmental changes can exceed changes observed over longer time periods  
204 (Siepielski et al. 2009, Bell 2010, Johnson 2011), and the results of the present study  
205 are most readily interpreted in this view. In Lobith, the proportion of yellow effectively  
206 unbanded (YeU), the character that responds strongly to visual (Cain and Sheppard  
207 1954) and thermal selection (Ozgo 2011, Ozgo & Schilthuizen 2012), was significantly  
208 lower in 1961 than in 1960, and significantly higher in 1962 than in 1961. However,  
209 there was statistically significant difference between 1960 and 1962. Consequently,  
210 when the 2010 sample is compared with 1962, no changes are observed; when the  
211 same data are compared with 1961, a significant increase in YeU is recorded and  
212 suggests an adaptive genetic shift. Similarly, a significant increase in YeU was  
213 observed in Allemansgeest between 1942 and 2010, also suggesting a strong adaptive  
214 response. However, no such change was observed between 1943 and 2010; a  
215 significant increase in YeU occurred between 1942 and 1943. Possibly the most striking  
216 is the case of Empe, where the re-survey encompassed the longest time interval. A

217 significant increase in YeU was observed between 1915 and 1951, but the frequency of  
218 YeU in 2010 was not significantly different from either 1915 or 1951. This suggests  
219 caution in comparing population states when sampling is carried out at long time  
220 intervals.

221

222 The results of this study might be very different if we had only one historic sample  
223 available from each locality. If we were comparing the results of the 2010 survey with  
224 the surveys of 1915 in Empe, 1943 in Allemansgeest, and 1962 in Lobith, the  
225 conclusion would be that no or very few observable changes occurred over those time-  
226 intervals. If however, by coincidence, we had available the data collected in Empe in  
227 1951, in Allemansgeest in 1942, and in Lobith in 1961, the conclusion might be that the  
228 observed changes are significant and consistent, and indicate a response of  
229 populations to contemporary climatic shift.

230

231 Obviously, when analyzing evolutionary changes, long time series of annual censuses  
232 are the most reliable and informative. In polymorphic land snails such a study was  
233 carried out by Cain et al. (1990), and subsequently re-analyzed by Bell (2010), and by  
234 Johnson (2011). This approach, however, is limited to previously initiated and  
235 maintained studies. These are regrettably few and generally they are incomparable as  
236 the time periods, geographic locations, and taxonomic groups involved are too far apart.

237

238 Another reliable approach is to compare subdivided populations when time of  
239 divergence is known (synchronic design *sensu* Hendry and Kinnison 1999). In *Cepaea*  
240 *nemoralis*, this approach allowed us to infer rapid adaptive changes in response to  
241 changed microclimates (Ozgo and Kinnison 2008, Ozgo 2011, Schilthuizen 2013). Time  
242 of divergence, however, is rarely known. Possibly the most promising approach is to  
243 use a group of populations all sampled and resampled at the same time (e.g. Ozgo and  
244 Schilthuizen 2012, Cameron et al. 2013). Here, the underlying assumption is that  
245 organisms in different populations respond similarly to the same larger-scale selection  
246 pressures; consistent changes over a number of populations are likely to reflect  
247 directional processes.

248

249 This study shows that conclusions drawn from comparisons of single populations  
250 resurveyed after long time intervals can be misleading. Consequently, other studies  
251 where populations were surveyed only a couple of times can possibly lead to results  
252 that are difficult to interpret (e.g. Silvertown et al. 2011). In sum, our data might  
253 contribute to the increasing recognition of large changes in the states of populations  
254 over very short time scales. Such changes may result from natural selection in response  
255 to biotic or abiotic factors (e.g. changing weather conditions and/or fluctuating selection  
256 by predators), and from random events. We also draw attention to some methodological  
257 concerns of selection studies, and point to the value of long time series in ecological  
258 and evolutionary research.

259

260

### 261 **Author Contributions**

262 MO conceived the study, did the field work, carried out the shell scoring for field and  
263 museum samples, did the initial analyses, and wrote drafts for sections of the paper.

264 MS conceived the study, did the field work, carried out the shell scoring for field and  
265 museum samples, and wrote the final draft of the paper.

266 NW did the field work, revised drafts of the paper, and prepared the figure.

267 LTS did the field work, refined the data analyses, and revised the draft of the paper.

268

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271 Mollusca collection for providing access to the historical specimens and for arranging  
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274 localities.

275

### 276 **References**

- 277 ○ Arthur W., D. Phillips & P. Mitchell, 1993. Long-term stability of morph frequency  
278 and species distribution in a sand-dune colony of *Cepaea*. *Proceedings of the*  
279 *Royal Society of London B*, 251: 159-163.
- 280 ○ Bell G. 2010. Fluctuating selection: the perpetual renewal of adaptation in  
281 variable environments. *Philosophical Transactions of the Royal Society B*, 365:  
282 87-97.
- 283 ○ Buswell J.M., A.T. Moles & S. Hartley, 2011. Is rapid evolution common in  
284 introduced plant species? *Journal of Ecology*, 99: 214-224.
- 285 ○ Cain A.J. & P.M. Sheppard, 1954. Natural selection in *Cepaea*. *Genetics*, 39: 89-  
286 116.
- 287 ○ Cain, A.J., L.M. Cook & J.D. Currey, 1990. Population size and morph frequency  
288 in a long-term study of *Cepaea nemoralis*. *Proceedings of the Royal Society of*  
289 *London B*, 240: 231-250.
- 290 ○ Cameron R.A.D. & B.M. Pokryszko, 2008. Variation in *Cepaea* populations over  
291 42 years: climate fluctuations destroy a topographical relationship of morph-  
292 frequencies. *Biological Journal of the Linnean Society*, 95: 53-61. DOI:  
293 10.1111/j.1095-8312.2008.01042.x
- 294 ○ Cameron R.A.D., L.M. Cook & J.J.D. Greenwood, 2013. Change and stability in a  
295 steep morph-frequency cline in the snail *Cepaea nemoralis* (L.) over 43 years.  
296 *Biological Journal of the Linnean Society*, 108: 473-483.
- 297 ○ Cheptou P.-O., O. Carrue, S. Rouifed & A. Cantarel, 2008: Rapid evolution of  
298 seed dispersal in an urban environment in the weed *Crepis sancta*. *Proceedings*  
299 *of the National Academy of Sciences, USA*, 105: 3796-3799.
- 300 ○ Cook L.M., 1998: A two-stage model for *Cepaea* polymorphism. *Philosophical*  
301 *Transactions of the Royal Society of London B*, 353: 1577-1593.  
302 doi:10.1098/rstb.1998.0311
- 303 ○ Goodhart C.B., 1973. A 16-year survey of *Cepaea* on the Hundred Foot bank.  
304 *Malacologia*, 14: 327-331.
- 305 ○ Hendry, A.P. & M.T. Kinnison, 1999. The pace of modern life: measuring rates of  
306 contemporary microevolution. *Evolution*, 53: 1637-1653.

- 307 ○ Johnson, M.S., 2011. Thirty-four years of climatic selection in the land snail  
308 *Theba pisana*. *Heredity*, 106: 741-748.
- 309 ○ Ożgo M., 2008. Current problems in the research of *Cepaea* polymorphism. *Folia*  
310 *Malacologica*, 16: 55-60.
- 311 ○ Ożgo M. & M.T. Kinnison, 2008. Contingency and determinism during convergent  
312 contemporary evolution in the polymorphic land snail, *Cepaea nemoralis*.  
313 *Evolutionary Ecology Research*, 10: 721-733.
- 314 ○ Ożgo M. 2011. Rapid evolution in unstable habitats: a success story of the  
315 polymorphic land snail *Cepaea nemoralis* (Gastropoda: Pulmonata). *Biological*  
316 *Journal of the Linnean Society*, 102: 251-262.
- 317 ○ Ożgo M. & M. Schilthuizen, 2012. Evolutionary change in *Cepaea nemoralis* shell  
318 colour over 43 years. *Global Change Biology*, 18: 74-81. doi: 10.1111/j.1365-  
319 2486.2011.02514.x
- 320 ○ Schilthuizen, M. 2013. Rapid, habitat-related evolution of land snail colour  
321 morphs on reclaimed land. *Heredity*, 110: 247-252.
- 322 ○ Schilthuizen, M., C. Vairappan, E. Slade, D. Mann & J. Miller, 2015. Specimens  
323 as primary data: museums and “open science”. *Trends in Ecology and Evolution*,  
324 20: 1-2.
- 325 ○ Siepielski, A.M., J.D. DiBattista & S.M. Carlson, 2009. It’s about time: the  
326 temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, 12: 1261-  
327 1276.
- 328 ○ Silvertown J., L. Cook, R. Cameron, M. Dodd, K. McConway, J. Worthington, P.  
329 Skelton, C. Anton, O. Bossdorf, B. Baur, M. Schilthuizen, B. Fontaine, H.  
330 Sattmann, G. Bertorelle, M. Correia, C. Oliveira, B. Pokryszko, M. Ożgo, A.  
331 Stalažs, E. Gill, Ü. Rammul, P. Sólymos, Z. Féher & X. Juan, 2011. Citizen  
332 science reveals unexpected continental-scale evolutionary change in a model  
333 organism. *PLoS ONE*, 6: e18927. doi:10.1371/journal.pone.0018927
- 334 ○ Suarez A.V. & N.D. Tsutsui, 2004. The value of museum collections for research  
335 and society. *BioScience*, 54: 66-74.
- 336 ○ Van Heurn, W.C. 1943. Stabiliteit van populaties van *Cepaea nemoralis* (L.).  
337 *Basteria*, 8: 59-63.

- 338      ○ Van Heurn, W.C. 1945. Stabiliteit van populaties van *Cepaea nemoralis* (L.),  
339      vervolg. *Basteria*, 9: 39-43.
- 340      ○ Wolda, H. 1969. Stability of a steep cline in morph frequencies of the snail  
341      *Cepaea nemoralis* (L.). *Journal of Animal Ecology*, 38: 623-635.

343

344

345 Figure 1. Proportional variation in morph frequencies for all sites in all years. A, Shell

346 ground colour morphs. B, Banding categories — Mid: mid-banded (00300);

347 Three: three-banded (00345); Five: five-banded (12345); Other: other banding

348 categories. C, Proportion of Yellow effectively unbanded (YeU). Numbers

349 indicate total count. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ . <sup>1</sup>Brown was excluded350 from all  $\chi^2$  tests except Empe from 1915, and Lobith 1962-2010. <sup>2</sup>Mid-banded351 was excluded from all Empe 1915 and Lobith 1960  $\chi^2$  tests. <sup>3</sup>Mid-banded was352 excluded from Empe 1951-2010  $\chi^2$  test.

353

355 Supplementary Data 1. R scripts for the analyses.

356

357 Supplementary Data 2. Full details of the original and the resampled collections from

358 Lobith, including a photo of the contemporary habitat.

359

360 Supplementary Data 3. Full details of the original and the resampled collections from

361 Empe, including a photo of the contemporary habitats.

362

363 Supplementary Data 4. Full details of the original and the resampled collections from

364 Allemansgeest, including a photo of the contemporary habitat.

365

366



**Figure 1**(on next page)

Proportional variation in morph frequencies for all sites in all years.

Figure 1. Proportional variation in morph frequencies for all sites in all years. A, Shell ground colour morphs. B, Banding categories — Mid: mid-banded (00300); Three: three-banded (00345); Five: five-banded (12345); Other: other banding categories. C, Proportion of Yellow effectively unbanded (YeU). Numbers indicate total count. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ . <sup>1</sup>Brown was excluded from all  $\chi^2$  tests except Empe from 1915, and Lobith 1962-2010. <sup>2</sup>Mid-banded was excluded from all Empe 1915 and Lobith 1960  $\chi^2$  tests. <sup>3</sup>Mid-banded was excluded from Empe 1951-2010  $\chi^2$  test.

