1   **Discrete or indiscrete? Redefining the colour polymorphism of the land snail**

2   ***Cepaea nemoralis***

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7   Running head: Redefining a snail colour polymorphism

8   Biologists have long tried to describe and name the different phenotypes that make

9   up the exuberant colour polymorphism of the land snail *Cepaea nemoralis*.

10   Traditionally, the view is that the ground colour is one of a few major colour classes,

11   either yellow, pink or brown, but in practise it is frequently difficult to distinguish the

12   colours, and consistently define different shades of the same colour. To understand

13   whether colour variation is continuous, and to investigate how the variation may be

14   perceived by an avian predator, we applied psychophysical models of colour vision

15   to shell reflectance measures. The main finding is that both achromatic and

16   chromatic variation are indiscrete, being continuously distributed over many

17   perceptual units, with the major axis of chromatic variation representing differences

18   in saturation, or purity of colour. Nonetheless, clustering analysis based on the

19   density of the distribution revealed three groups, roughly corresponding to human-

20   perceived yellow, pink and brown shells. There is also large-scale geographic

21   variation between these morphs across Europe, and some covariance between shell

22   colour and banding patterns. Although further studies are necessary to understand

23   the evolutionary origins and impact of natural selective upon this variation, the

24   observation of continuous variation in colour is intriguing, given that the underlying

25   supergene that determines colour should prevent phenotypes from "dissolving" into

26   continuous trait distributions.

28 Throughout the past century, the study of animal colour has been critical in making

29 progress in understanding the principles of biology, especially with respect to

30 genetics and evolution (McKinnon and Pierotti 2010; McLean and Stuart-Fox 2014;

31 Cuthill et al. 2017; San-Jose and Roulin 2017). For instance, early studies on the

32 inheritance of colour traits were important in establishing an understanding of basic

33 Mendelian genetics (Wheldale 1907; Staples-Browne 1908). Subsequently, studies

34 of the distribution and predation of colour morphs have and continue to shape our

35 understanding of how natural and sexual selection operate in wild populations

36 (Hugall and Stuart-Fox 2012; Dale et al. 2015; Delhey et al. 2017). Most recently,

37 candidate gene and latterly genomic approaches have been used to identify the

38 underlying genes that determine the colour differences (references in Hoekstra 2006;

39 McLean and Stuart-Fox 2014; San-Jose and Roulin 2017).

40   For practical reasons, many of these prior studies have taken advantage of

41 traits that exhibit relatively simple, discrete variation and straightforward inheritance

42 patterns, but this risks missing the extraordinary variation of life forms and colour

43 traits. It is also likely that in nature discrete variation is the exception rather than the

44 rule – and this is becoming more evident as researchers increasingly use

45 instrumentation to measure colour (Montgomerie 2006), rather than being obliged to

46 bin types into human-defined categories.

47   Historically, two of the most important animals in studying colour

48 polymorphism have been the peppered moth *Biston betularia* and the grove snail

49 *Cepaea nemoralis* and its sister taxon, *C. hortensis* (collectively "*Cepaea*", the

50 preferred common name), because individuals are relatively easy to collect and

51 study, and the colour morphs show straightforward inheritance. However, while

52 ongoing and long-term studies on these animals continue to provide compelling

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53   evidence for the fundamental role of natural selection in promoting and maintaining

54   variation in natural populations, as well as the impact of modern-day habitat change

55   (Silvertown et al. 2011; Cook et al. 2012), progress in understanding the precise

56   mechanism of the polymorphisms has diverged in the two systems.

57   In the peppered moth, the precise mutation that determines the colour

58   differences was reported to be in a known patterning gene (van't Hof et al. 2016). In

59   contrast, an understanding of the 'exuberant' (Franks and Oxford 2009)

60   polymorphism of *Cepaea* – whether yellow, pink or brown shells and zero to five

61   bands – has stalled since the 1970s. In part, this may be a reaction to the question of

62   Jones *et al.* (1977) on whether the *Cepaea* polymorphism is "a problem with too

63   many solutions?" Actually, the intention of that work was to emphasise the perfect

64   case study provided by *Cepaea*; as simple explanations for phenotypic variation are

65   the exception, Jones *et al.* were making the point that it is important to study

66   organisms for which polymorphism may be explained by a variety of processes,

67   precisely because they are more realistic. Some 40 years later, this comment is still

68   relevant, given that genomic technologies and DNA sequence analyses should allow

69   us to uncover the relative contributions of each of these processes to contemporary

70   diversity – but it is nonetheless understandable that most study systems are still

71   selected because of their relative simplicity.

72   Despite a general lack of progress, the *Cepaea* polymorphism retains

73   excellent potential as a model system in evolutionary biology. Previous studies have

74   laid the foundations for future progress, including exploiting many long term studies

75   (Cook et al. 1999; Davison and Clarke 2000; Silvertown et al. 2011; Cameron and

76   Cook 2012; Ożgo and Schilthuizen 2012; Cameron et al. 2013; Schilthuizen 2013;

77   Cook 2014; Ozgo et al. 2017), increasing understanding of the pigments and shell

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78   proteome (Mann and Jackson 2014; Williams 2017) and especially, using new

79   genomic methods to identify the genes involved (Richards et al. 2013; Kerkvliet et al.

80   2017). Of particular interest here, we note (as do others, Surmacki et al. 2013) that

81   there is now a pressing need to quantify objectively the polymorphism of *Cepaea*

82   shells, and to understand how this is perceived by an avian predator, because only

83   then can we properly understand how the polymorphism is maintained. The specific

84   problem is that in the past *Cepaea* shell colours have usually been treated as one of

85   three or more distinct classes (e.g. Cain and Sheppard 1954) – yellow, pink or brown

86   – partly due to a lack of objective measures of colour, especially those that can be

87   used in the field and between different observers and contexts (Cain et al. 1960;

88   Cain et al. 1968; Jones et al. 1977). There has been also the significant issue that

89   human perception of colour is not necessarily objective or the same as that of an

90   avian predator (Surmacki et al. 2013).

91   Now that objective methods of measuring and analysing colour are widely

92   available (Endler 1990; Montgomerie 2006; Maia et al. 2013; Delhey et al. 2015) and

93   easy to use, at least in a controlled laboratory setting, we set out to measure

94   quantitatively the ground colour of *Cepaea* shells, and so to define the nature of the

95   polymorphism. Specifically, by measuring the shell colour of snails collected across

96   the breadth of the European distribution, we used psychophysical models of colour

97   vision to assess how chromatic variation is perceived by birds (but not categorised;

98   Caves et al. 2018).

99   Previously, Surmacki *et al.* (2013) used quantitative measures of colour on

100   relatively few individuals to assess how shells match to various backgrounds. Here,

101   we investigate the extent to which the distribution of snail shell colour is continuous

102   along the main axes of chromatic variation, using more than a 1000 individuals and

103   Gaussian finite mixture modelling (Scrucca et al. 2016) to test whether colours fall

104   into clusters in multivariate space. We also aimed to understand if quantitative

105   measures on a relatively small sample of shells can describe – rather than explain –

106   geographic patterns in colour morph frequency across Europe, as others have done

107   in much larger qualitative surveys.

108   The findings have significance for understanding the *Cepaea* polymorphism,

109   and the nature of the selection that acts upon it, as well as more generally

110   highlighting the need to measure colour objectively in other systems, before being

111   able to test for possible explanations.

112   *Methods*

113   Data collection

114   Individual *C. nemoralis* snails were mainly gathered opportunistically by volunteer-

115   led collection and field trips across Europe (Grindon and Davison 2013). Snails were

116   frozen upon arrival at the University of Nottingham, subsequently thawed and the

117   body extracted from the shell. The ground colour and banding of the shell was then

118   scored qualitatively by an experienced person (A.D.) and a student, as either yellow

119   (Y), pink (P) or brown (B), and unbanded (O), mid-banded (M), or all other banding

120   patterns (B, usually five-banded). Subsequent statistical analyses were carried out at

121   the level of the individual and the level of the population (sample site). So that we

122   could compare broad-scale patterns across Europe, larger groups were also used –

123   individual sample sites were therefore grouped into one of six larger groups (Table

124   S1; Figure 1).

125   An Ocean Optics spectrometer (model USB2000+UV-VIS-ES) and a light

126   source (DT-MINI-2-GS UV-VIS-NIR) were used to measure individual reflectance

127   spectra of shells, using a WS-1 diffuse white reflectance standard to set the baseline

128   light spectrum (Teasdale et al. 2013; Taylor et al. 2016), and complete darkness to

129   set the dark spectrum standard. Reflectance measurements were taken on the

130   underside of the dried shell, because it was usually the least damaged region, least

131   exposed to sunlight, and well away from any bands. Point samples were taken for

132   each shell at a 45° incident angle, 2 mm from the shell. Individual shells were

133   measured three times, non-consecutively, with the software recalibrated against light

134   standards every 2-5 measurements. Readings were collected using Ocean Optics

135   SpectraSuite v. 2.0.162 (software settings: integration time 750msec, boscar width 5,

136   scans to average 10); then the raw data smoothed and binned into 5 nm categories

137   using Pavo version 0.5-6 (Maia et al. 2013).

138   Analysing chromatic and achromatic variation

139   We used the framework provided by Delhey et al. (2015) to analyse the reflectance

140   spectra. In this framework, a psychophysical model of colour vision (Vorobyev and

141   Osorio 1998; Vorobyev et al. 1998) is used to assess whether chromatic differences

142   between reflectance spectra exceed a discrimination threshold, or 'just noticeable

143   difference' (JND), which can be perceived by a receiver, such as an avian predator.

144   The key to these models lies with the degree to which a particular combination of

145   reflectance and illuminant spectra stimulate each of the different photoreceptors in

146   the retina. In birds, these photoreceptors are the four single cones used for colour

147   vision, which are sensitive to long (L), medium (M), short (S), and very short (VS)

148   wavelengths of light (Cuthill 2006).

149   To analyse chromatic variation, the quantum catches for each cone type were

150   converted into three chromatic coordinates (x, y and z), where Euclidean distances

151   between points reflect perceptual differences, using the formulas of Cassey *et al.*

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152   (2008). As there are no data for the song thrush, *Turdus philomelos*, which is the

153   main predator of *Cepaea*, we used sensitivity functions for the closest available

154   relative, the blackbird *Turdus merula* (Hart et al. 2000; Hart 2001), namely cone

155   proportions of VS: 0.528, S: 0.904, M: 1.128, and L: 1, sensitivity functions of 373,

156   461, 543 and 603, respectively. The analysis assumed that the L cone has a noise-

157   to-signal ratio of 0.05, so that the ratios for the other cones were VS: 0.0688, S:

158   0.0526 and M: 0.0471. The irradiance spectrum of 'standard daylight' (d65) was

159   used for the main analyses. However, analyses were also run for 'woodland shade'

160   to understand the influence of illuminant on avian perception of colour (Vorobyev et

161   al. 1998).

162   To identify the main axes of chromatic variation, we carried out a Principal

163   Components Analysis (PCA) on the chromatic coordinates (x, y and z), preserving

164   the perceptual distances (JNDs) by using a covariance matrix rather than a

165   correlation matrix (Delhey et al. 2015). To understand whether there are potential

166   clusters within the chromatic coordinate data, Gaussian mixture modelling was

167   carried out using Mclust 5.3 in R version 3.3.3 (Scrucca et al. 2016). A number of

168   models were compared, each of which assumed a different number of clusters (from

169   1 to 10), normally distributed in multivariate chromatic space. Several classes of

170   model were considered, each with a different assumption about the homogeneity of

171   variance and orientation among clusters. The best fitting model was then determined

172   as the one with highest Bayesian Information Criteria (BIC), with significant

173   differences determined using a bootstrap approach.

174   The methods of Delhey et al. (2015) were also used to assess achromatic

175   variation. In birds, sensitivity to achromatic cues is supposed to be mediated by

176   double cones which have the same pigment as L cones in birds but different oil

177 droplets, so have a wider sensitivity range. Values of achromatic contrast were

178 therefore estimated, again in JNDs, by computing achromatic contrast between each

179 reflectance spectrum and a reference (a very low value of double cone quantum

180 catch, 0.001), corresponding to a dark spectrum, and using the same noise-to-signal

181 ratio.

182 Analysis of morph frequencies

183 We investigated evidence for effects of location and banding on the likelihood that a

184 snail belonged to a particular morph, using generalised linear mixed effects models

185 (GLMMs) with binomial errors. Each morph was considered separately, with each

186 snail to be scored as belonging to the focal morph (1) or not (0). The three analyses

187 are not independent, since each snail can only belong to one morph. Banding

188 pattern was fitted as a fixed factor, whilst the effect of geographic location was

189 examined at three spatial scales. Variation in morph frequency at a local level was

190 modelled with random effect for site. Variation at a regional level was considered by

191 fitting a fixed effect of geographic region. Finally, continental scale variation was

192 modelled by looking for fixed linear and quadratic effects of latitude and longitude.

193 The fact that region and latitude/longitude are partially collinear was reflected in the

194 model-fitting procedure. We first fitted a saturated model with all main effects, except

195 for region, and their two-way interactions (excluding interactions involving quadratic

196 effects). Then, fixed terms were removed in a stepwise fashion, testing the effect of

197 deletion using likelihood ratio tests, until only significant terms remained. Effects of

198 latitude/longitude were then substituted with an effect of region and we compared the

199 Akaike Information Criterion (AIC) of the resulting models, to test if region was better

200 at capturing any large-scale geographic variation. Testing random effects in

201 generalised linear mixed models is problematic, so we compared the AIC of the

202   saturated GLMM with that of a generalised linear model without the random term for

203   site to provide an approximate test of the importance of site.

204   *Results*

205   Variation in colour

206   We measured the individual reflectance spectra of 1172 shells, mainly collected from

207   across Europe (Table S1; Figure 1) and then transformed them into visual space

208   coordinates, xyz. To visualise this chromatic variation, and the relationship with

209   human-scored and Mclust-defined colour categories (below), the xyz coordinates

210   were plotted in visual colour space (Figure 2). There were no obviously discrete

211   groups.

212   A PCA on the xyz coordinates showed a first axis which explained 87% of

213   chromatic variation. PC1 had a moderate positive loading for x (0.61), and a

214   moderate negative loading for y (-0.64) and z (-0.46). Two further axes explained

215   11% and 2% of the variation, the second having a positive loading on all axes (0.75,

216   0.28, 0.61, respectively), and the third a mixture (-0.26, -0.71, 0.65). The range of

217   observed variation on each axis was considerable: 41, 22 and 8 JNDs for x, y and z

218   respectively (Figure 2). Plotting the average normalized reflectance spectra for each

219   quartile of each principal component showed how the three PC axes correspond to

220   chromatic variation (Figure 3). Variation along PC1 represents relatively high

221   stimulation of L cones and lesser stimulation of S cones, relative to M cones.

222   Variation in PC2 showed relatively higher stimulation of L cones and lesser

223   stimulation of M cones. PC3 showed relatively high stimulation of the M cones

224   compared to lesser stimulation of the S and L cones. Only PC1 showed any

225   differences in the VS region but the shells barely reflected in the UV.

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226   To investigate whether snail shells cluster in chromatic space, and whether

227   observed clusters correspond to human-scored qualitative colour morphs, Gaussian

228   finite mixture modelling was applied to the xyz visual space coordinates. The best

229   model (VVV, ellipsoidal, varying volume, shape, and orientation; BIC -15727.3; $P <$

230   0.001 compared 2nd best model) recovered three clusters, roughly corresponding to

231   human-scored yellow (46%, n=539), pink (44%, n=511) and brown (10%, n=122)

232   (Table 1). The next best fitting model also recovered three clusters (VEV; BIC -

233   15749.3; $P < 0.001$ compared with 3rd best model) and the third recovered four

234   clusters (EEV; BIC -15755.5; the 4th cluster contained only 16 individuals).

235   Comparing human-scored (A.D.) and Mclust-defined groups, the overall

236   concordance was good at 76% (Table 1), with a similar error rate (75%) for the

237   student group. The highest proportion of discordant scores were human-scored

238   yellow shells that Mclust classed as pink (10% for A.D.; 12% for student group), with

239   the other major discrepancies being human-pink classed as Mclust-brown (8%), and

240   human-yellow classed as Mclust-pink (4%). The main difference was that human-

241   scoring reported relatively few brown shells (n=37), whereas the same group in

242   Mclust was larger (n=122). With misclassifications adjusted relative to the total

243   number of each Mclust shell type, 81% of the brown group were in a different

244   human-scored group (74% pink, 7% yellow), compared to 26% of the pinks (3%

245   brown 23% yellow) and just 8% of yellows (8% pink, 0% brown). Thus, while the

246   overall correspondence between human and Mclust scoring of shell colour was

247   good, the yellows were scored accurately (92%), pinks less so (74%) and brown

248   poorly (19%).

249   Plots of human-scored colours along the three PCs (Supplementary Movie 1)

250   and Mclust-categories were concordant with the above analyses (Figures 2, 4;

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251   Supplementary Movie 2). Broadly, PC1 did not separate different human-perceived

252   colours or categories of shell, but instead mainly represents differences in saturation,

253   or purity of colour, between individuals (Figure 4). PC2 separated brown from yellow

254   and pink, and PC3 broadly separated all three colours, yellow, pink and brown.

255   The above analyses were repeated using woodland shade rather than

256   standard daylight. The main difference was that while Mclust again recovered three

257   groups, brown shells were more common (14%, n=168), with fewer pinks (40%,

258   n=474) and approximately the same number of yellows (45%, n=530).

259   Finally, achromatic variation was also considerable, varying over more than

260   100 JNDs, and without any obvious differences between Mclust-defined colour

261   morphs (Supplementary Figure 1).

262   Geographic variation between morphs

263   Large-scale geographic variables (latitude, longitude and region) had significant

264   effects on the probability that a snail was pink or yellow, but not the probability that a

265   snail was brown (Table 2). Pink morphs were significantly less common at mid-

266   latitudes (Figure 5). Snails with more than one band (B) and those which were mid-

267   banded (M) were more likely to be pink in the west, while unbanded (O) snails were

268   more likely to be pink in the east (Figure 5). In contrast, yellow snails were less

269   common at high latitudes, and were affected by an interaction between longitude and

270   banding which was the reciprocal of that seen in pink snails (Figure 6). Morph

271   frequencies also varied at a local level: a saturated mixed model including the

272   random effect of site was much better (Brown AIC = 443.7; Pink AIC = 868.9; Yellow

273   AIC = 852.8) than an equivalent model without the random effect (Brown AIC =

274   646.4; Pink AIC = 1407.9; Yellow AIC = 1416.2). Banding was associated with colour

275 morph in various ways. In addition to the interaction between banding and longitude

276 in pink and yellow snails mentioned above, unbanded snails (O) were generally more

277 likely to be brown (14% of all unbanded snails), than snails that were mid-banded

278 (M; 11.6%) or had several bands (B; 5.8%).

279 *Discussion*

280 By measuring the ground colour of *Cepaea nemoralis* shells collected across the

281 breadth of the European distribution, we used psychophysical models of avian vision

282 to understand how the shell colour may be perceived by birds, and to describe how

283 this varies in geographic space, and with respect to other characters such as

284 banding. The findings have significance for understanding the *Cepaea*

285 polymorphism, and the nature of the selection that acts upon it, as well as more

286 generally highlighting the need to objectively measure colour variation in other

287 systems before beginning to test for possible explanations.

288   Broadly, we found that both chromatic (Figure 2) and achromatic variation

289 (Supplementary Figure 1) is considerable, occurring over many perceptual units

290 (JNDs). If this variation, both within and among human-perceived colour morphs,

291 affects prey detection or identification by avian predators, then the presumption is

292 that the polymorphism must be impacted by natural selection. The current available

293 evidence suggests that animals in general use chromatic and achromatic signals for

294 separate tasks, for example, using achromatic signals to identify the location, shape

295 and motion of objects, while chromatic signals identify surface quality (Osorio and

296 Vorobyev 2005). However, while this is also likely the case for avian predators,

297 specific experimental evidence from birds is sparse (Osorio et al. 1999; Kang et al.

298 2015; White and Kemp 2016).

299   In our analysis, we found that chromatic variation in shells is continuously

300   distributed in visual space, meaning that there are no wholly discrete colours (Figure

301   2). Perhaps surprisingly, we found that the most variable chromatic axis (PC1; 87%)

302   that would be visible to a bird reflects the degree of saturation, or purity of colour.

303   Axes separating human-perceived colours showed less variation, PC2 (11%)

304   separating brown from yellow/pink, and PC3 (2%) broadly separating yellow, pink

305   and brown.

306   Despite the lack of discrete colours, density-based clustering recovered three

307   main shell types, which roughly correspond to human-perceived yellow, pink and

308   brown (Table 1; Figure 3). Brown shells were more common according to the

309   objective analysis than perceived by humans, with the frequency higher again when

310   using woodland shade as an illuminant. Therefore, prior studies that (necessarily)

311   used changes in frequencies of human-perceived colours to understand natural

312   selection on snail shells may have missed a significant part of the picture – not only

313   may birds use both achromatic and chromatic cues to differentiate morphs, but they

314   should also be able to perceive chromatic differences to a much finer precision than

315   a simple trivariate yellow, pink or brown categorisation that humans are obliged to

316   use in qualitative surveys. Of course, this does not mean that birds react to the many

317   morphs equally – it is possible that they categorically perceive a continuous variable

318   (Caves et al. 2018). Further investigations are needed, especially using a bird such

319   as the song thrush.

320   The effects of geographic location and banding pattern on variation in the

321   reflectance spectrum of snails were also examined, the initial aim being to develop

322   methods to *describe* variation, rather to *explain* it (e.g. by looking for correlations with

323   environmental variables, putative selective agents, etc., as others have done;

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324   Silvertown et al. 2011). Generally, we found that geographic variables (latitude,

325   longitude and region) and banding are generally associated with different

326   frequencies of the three traditional colour morphs, with the main directional trend

327   being that yellow snails are most common at mid-latitudes, as was found in much

328   larger studies (Jones et al. 1977; Silvertown et al. 2011). Similarly, as previously

329   reported (Cain et al. 1960), epistasis meant that unbanded snails (O) were generally

330   more likely to be brown, and banded snails (B) were less likely to be brown.

331   Therefore, by establishing a method for quantitatively measuring colour, and

332   showing that a relatively small sample can be used to infer wide geographic patterns,

333   this work provides a baseline for further studies on the polymorphism.

334   *Discrete or indiscrete?*

335   Laboratory crosses in the past have revealed that the variation in the *Cepaea* shell

336   phenotype is predominantly controlled by a 'supergene', which in a recent definition

337   is a *genetic architecture involving multiple linked functional genetic elements that*

338   *allows switching between discrete, complex phenotypes maintained in a stable local*

339   *polymorphism* (Thompson and Jiggins 2014; Llaurens et al. 2017). This meaning fits

340   with the traditional view – and the classical 'Fordian' theory of polymorphism (Ford

341   1964) – that the ground colour of the shell is one of three more or less discrete

342   colour classes, either yellow, pink or brown, and indeed, is part of the reason that

343   *Cepaea* snails became a well-studied system. However, while scoring the shell

344   colour into different, discrete types is straightforward in offspring of individual crosses

345   in the lab, the acknowledged reality is that it is sometimes difficult to classify shells

346   consistently (e.g. see Table 1), especially when they are apparently intermediate in

347   form.

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348   Now, in our study, we have shown definitively that the colour polymorphism of

349   *Cepaea nemoralis* is not discrete (Figures 2, 4). This finding emphasises the specific

350   practical problem for projects collecting and using shell polymorphism data,

351   especially those based entirely in the field and using citizen science (e.g. Evolution

352   Megalab; Silvertown et al. 2011). However, it also illustrates a more general

353   problem: if we do not have a precise definition of the *Cepaea* polymorphism and an

354   understanding of the underlying genetic control, then how can we claim to

355   understand the evolutionary and ecological factors that maintain colour variation?

356   Mathematical modelling is one method that can be used to explore the

357   evolution of polymorphism, and of most relevance to this work, the circumstances

358   that may or may not lead to discrete phenotypes. Historically, the argument of Ford

359   was that despite the fact that supergenes may appear as Mendelian loci, they were

360   actually rather complicated arrangements of several loci that are effectively

361   prevented from being broken up by recombination under most normal

362   circumstances. Thus, in both colour polymorphism in general (e.g. in side-blotched

363   lizards; Sinervo and Lively 1996) and specifically relating to supergenes (e.g. in

364   butterfly mimicry rings; Joron et al. 2011; Kunte et al. 2014), the distinctiveness of

365   the morphs is a central feature of the genetic control; the genetic architecture

366   specifically prevents phenotypes from "dissolving" into continuous trait distributions

367   (Ford 1964).

368   Much of the existing research has therefore begun from the premise of

369   understanding how evidently discrete types come about, and thus give insight into

370   the adaptive evolution of genome structure (Cuthill et al. 2017). In simulations it has

371   been have shown that natural selection tends to favour lowered recombination when

372   intermediate genotypes are at a disadvantage; unlinked loci modify the phenotype to

373   adapt to local conditions (e.g. to a local Batesian model butterfly; Charlesworth and

374   Charlesworth 1975b, a; Llaurens et al. 2017). More recently, and perhaps most

375   directly relevant to understanding the *Cepaea* polymorphism, Kopp and Hermisson

376   (2006) devised a model to investigate the evolution of a quantitative trait under

377   frequency-dependent disruptive selection. Their finding was that over generations

378   most of the genetic variation tends to concentrate on a small number of loci.

379   The historic background is perhaps part of the reason that most of the recent

380   progress in understanding supergenes has mainly come from species or systems

381   that show simple, wholly discrete phenotypes, for example in butterfly mimicry rings

382   (Joron et al. 2011), or heterostylous plants (Li et al. 2016). However, in *Cepaea* there

383   are many colour morphs, such that colour variation is quantitative and due to a

384   supergene; in other species such as the guppy *Poecilia* and the cichlid

385   *Labeotropheus*, the inheritance of often considerable colour variation is due to

386   several loci, some sex-linked and others not (Tripathi et al. 2009; Thompson and

387   Jiggins 2014; Wellenreuther et al. 2014). Thus, developing theory on the impact of

388   negative frequency-dependent (apostatic) selection must be able to account for

389   these complexities, including those where supergenes are absent and variation is

390   quantitative, otherwise there is a risk that models will simply reaffirm what we already

391   know.

392   In one recent model, it was shown that crypsis and apostatic selection

393   together may act to maintain a large number of morphs within a population, and in

394   another apostatic selection was shown to maintain variation between similar species

395   (Franks and Oxford 2011, 2017). In another more recent study, a simulation was

396   used to explore the influence of predator perspective, selection, migration, and

397   genetic linkage on colour allele frequencies. The relative sizes of predator and prey

398 home ranges can result in large differences in morph composition between

399 neighbouring populations (Holmes et al. 2017). Finally, in an empirical study blue

400 jays *Cyanocitta cristata* searched for digital moths on mixtures of dark and light

401 patches at different scales of heterogeneity. It was found that complex backgrounds

402 with many moth-like features elicited a slow, serial search that depended heavily on

403 selective attention. The result was increased apostatic selection, producing a broad

404 range of moth phenotypes (Bond and Kamil 2006). All of these circumstances may

405 apply to the *Cepaea* colour polymorphism.

406   Overall, there is an open debate – but little empirical data – on how the

407 relative heterogeneity of the environment/substrate, density, distance or motion may

408 influence the selection for crypsis or negative frequency dependence (Cuthill et al.

409 2017; Barnett et al. 2018). As Surmacki *et al.* (2013) summarised, if heterogeneous

410 areas consist of large patches of diverse habitats then this may promote the

411 evolution of specialist morphs through selection for crypsis, producing a few distinct

412 or specialist morphs, each more or less well matched to the coloration of the

413 preferred habitat type (Endler 1978; Bond 2007). If instead there are a mixture of

414 small microhabitats, apostatic selection is more likely to result in multiple morphs that

415 may be equally cryptic in all "grains" of the habitat. This is because in such

416 circumstances, predators use search images of the most common morph, and this

417 can lead to frequency-dependent selection.

418 *Supergenes return*

419 In contrast to a relative paucity of field data, and a relatively lack of progress in

420 establishing baseline theory, advances in DNA sequencing technology have meant

421 that knowledge on the genetics of colour polymorphism is advancing rapidly. As

422 hypothesised, in the still relatively few supergenes that have been fully

18

423   characterised, the discrete phenotypes are maintained due to close physical

424   proximity of the gene(s) and/or tight linkage (Joron et al. 2011; Kunte et al. 2014;

425   Gautier et al. 2018).

426   In comparison, a few more general studies on colour polymorphism, rather

427   than on supergenes specifically, have begun to reveal the extent of phenotypic

428   variation, and whether discrete or indiscrete. For example, reflectance spectra have

429   been used to show that even though humans perceived the colour variation in the

430   eggs of African cuckoo finch *Anomalospiza imberbis* as falling into discrete

431   categories, the variation was actually continuous (Spottiswoode and Stevens 2010,

432   2011). Similarly, tawny dragon lizard *Ctenophorus descresii* does have discrete

433   colour morphs, but there is still considerable variation within each morph (Teasdale

434   et al. 2013). Further quantitative studies in other lizards in which colour

435   polymorphism has traditionally been treated as qualitative are also increasingly

436   showing that there are significant overlaps in colour (Cote et al. 2008; Vercken et al.

437   2008; Paterson and Blouin-Demers 2017).

438   In *Cepaea nemoralis* snails, the colour and banding elements of the

439   supergene have been mapped (Richards et al. 2013) but we remain ignorant of the

440   underlying genetics and the precise nature of the selection that acts upon the

441   polymorphism. For instance, models of supergene evolution require that intermediate

442   phenotypes are disadvantaged – this makes sense with respect to Batesian mimics

443   or distylous flowers – but in snails a rare intermediate might be at an advantage, due

444   to apostatic selection. At the molecular level, one scenario is that the extreme and

445   effectively continuous colour variation of the shells is due to a corresponding high

446   number of colour alleles within the supergene. An alternative scenario is that there

447   actually relatively few colour alleles, with much of the chromatic variation due to

448   effects of other modifying loci (Charlesworth and Charlesworth 1975b). A final

449   consideration is that while colour variation might be continuous across a grand

450   geographic scale, if most local populations are founded by few individuals, then local

451   variation might be discrete, which is all that matters from a selective point of view.

452   This is more likely to be the case when both colour and banding are considered as

453   the visible phenotype, especially since they are frequently in linkage disequilibrium

454   (Cook 2017).

455   Overall, by establishing a method for quantitatively measuring colour, this

456   work provides a baseline for further studies on the polymorphism, both from the

457   perspective of understanding the nature of selection, and ultimately, also the genes

458   involved. To reconcile and test competing theories with the empirical observations,

459   the next steps must be to identify the component parts and evolutionary origins of

460   the supergene in *C. nemoralis*, develop a model of frequency-dependent selection,

461   and further understand how birds react to specific elements of the chromatic

462   variation. When all of these findings are brought together, only then can we begin to

463   understand the evolutionary and ecological factors that maintain this "problem with

464   too many solutions." Whatever the final outcome, there is no risk that *Cepaea* snails

465   will be relegated to "other adaptive polymorphism" (Thompson and Jiggins 2014),

466   especially because, as Jones *et al.* (1977) suggested, it is important to study

467   organisms for which polymorphism may be explained by a variety of processes,

468   precisely because they are more realistic.

469   Supplementary material

470   Supplementary Movies 1, 2. Supplementary Figure 1. Supplementary Table 1.

471   Funding

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480   Data archiving

481   Raw reflectance data will be included with the manuscript or uploaded to Dryad upon

482   acceptance of the manuscript.

483   *Literature cited*

484   Barnett, J. B., C. Michalis, N. E. Scott-Samuel, and I. C. Cuthill. 2018. Distance-
485   dependent defensive coloration in the poison frog *Dendrobates tinctorius*,
486   Dendrobatidae. Proc Natl Acad Sci USA 115:6416-6421.

487   Bond, A. B. 2007. The evolution of color polymorphism: crypticity searching images,
488   and apostatic selection. Annu Rev Ecol Evol Syst 38:489-514.

489   Bond, A. B. and A. C. Kamil. 1998. Apostatic selection by blue jays produces
490   balanced polymorphism in virtual prey. Nature 395:594-596.

491   Bond, A. B. and A. C. Kamil. 2002. Visual predators select for crypticity and
492   polymorphism in virtual prey. Nature 415:609-613.

493   Bond, A. B. and A. C. Kamil. 2006. Spatial heterogeneity, predator cognition, and the
494   evolution of color polymorphism in virtual prey. Proc Natl Acad Sci USA
495   103:3214-3219.

496   Cain, A. J., J. M. B. King, and P. M. Sheppard. 1960. New data on the genetics of
497   polymorphism in the snail *Cepaea nemoralis* L. Genetics 45:393-411.

498   Cain, A. J. and P. M. Sheppard. 1954. Natural selection in *Cepaea*. Genetics 39:89-
499   116.

Cain, A. J., P. M. Sheppard, and J. M. B. King. 1968. Studies on *Cepaea*. I. Genetics of some morphs and varieties of *Cepaea nemoralis* (L). Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 253:383-&.

Cameron, R. A. D. and L. M. Cook. 2012. Habitat and the shell polymorphism of *Cepaea nemoralis* (L.): interrogating the Evolution Megalab database. J Molluscan Stud 78:179-184.

Cameron, R. A. D., L. M. Cook, and J. J. D. Greenwood. 2013. Change and stability in a steep morph-frequency cline in the snail *Cepaea nemoralis* (L.) over 43 years. Biol J Linn Soc 108:473-483.

Cassey, P., M. Honza, T. Grim, and M. E. Hauber. 2008. The modelling of avian visual perception predicts behavioural rejection responses to foreign egg colours. Biol Lett 4:515-517.

Caves, E. M., P. A. Green, M. N. Zipple, S. Peters, S. Johnsen, and S. Nowicki. 2018. Categorical perception of colour signals in a songbird. Nature.

Charlesworth, D. and B. Charlesworth. 1975a. Theoretical genetics of Batesian mimicry I. Single-locus models. J Theor Biol 55:283-303.

Charlesworth, D. and B. Charlesworth. 1975b. Theoretical Genetics of Batesian Mimicry II. Evolution of supergenes. J Theor Biol 55:305-324.

Cook, L. M. 2014. Morph frequency in British *Cepaea nemoralis*: what has changed in half a century? J Molluscan Stud 80:43-46.

Cook, L. M. 2017. Reflections on molluscan shell polymorphisms. Biol J Linn Soc 121:717-730.

Cook, L. M., R. H. Cowie, and J. S. Jones. 1999. Change in morph frequency in the snail *Cepaea nemoralis* on the Marlborough Downs. Heredity 82:336-342.

Cook, L. M., B. S. Grant, I. J. Saccheri, and J. Mallet. 2012. Selective bird predation on the peppered moth: the last experiment of Michael Majerus. Biol Lett 8:609-612.

Cote, J., J. F. Le Galliard, J. M. Rossi, and P. S. Fitze. 2008. Environmentally induced changes in carotenoid-based coloration of female lizards: a comment on Vercken et al. J Evol Biol 21:1165-1172.

Cuthill, I. C. 2006. Color perception. Pp. 3-40 *in* G. E. Hill, and K. J. McGraw, eds. Bird coloration. Cambridge (MA): Harvard University Press.

Cuthill, I. C., W. L. Allen, K. Arbuckle, B. Caspers, G. Chaplin, M. E. Hauber, G. E. Hill, N. G. Jablonski, C. D. Jiggins, A. Kelber, J. Mappes, J. Marshall, R. Merrill, D. Osorio, R. Prum, N. W. Roberts, A. Roulin, H. M. Rowland, T. N. Sherratt, J. Skelhorn, M. P. Speed, M. Stevens, M. C. Stoddard, D. Stuart-Fox, L. Talas, E. Tibbetts, and T. Caro. 2017. The biology of color. Science 357.

539 Dale, J., C. J. Dey, K. Delhey, B. Kempenaers, and M. Valcu. 2015. The effects of
540   life history and sexual selection on male and female plumage colouration.
541   Nature 527:367-370.

542 Davison, A. and B. Clarke. 2000. History or current selection? A molecular analysis
543   of 'area effects' in the land snail *Cepaea nemoralis*. Proc R Soc Lond B Biol
544   Sci 267:1399-1405.

545 Delhey, K., V. Delhey, B. Kempenaers, and A. Peters. 2015. A practical framework
546   to analyze variation in animal colors using visual models. Behav Ecol 26:367-
547   375.

548 Delhey, K., B. Szecsenyi, S. Nakagawa, and A. Peters. 2017. Conspicuous plumage
549   colours are highly variable. Proc R Soc Lond B Biol Sci 284.

550 Endler, J. 1978. A predator's view of animal color patterns. Pp. 319-364 *in* M. K.
551   Hecht, W. C. Steere, and B. Wallace, eds. Evolutionary biology. Plenum
552   Press, New York.

553 Endler, J. A. 1990. On the measurement and classification of color in studies of
554   animal color patterns. Biol J Linn Soc 41:315-352.

555 Ford, E. B. 1964. Ecological Genetics. Methuen, London.

556 Franks, D. W. and G. S. Oxford. 2009. The evolution of exuberant visible
557   polymorphisms. Evolution 63:2697-2706.

558 Franks, D. W. and G. S. Oxford. 2011. The interrelationship between crypsis and
559   colour polymorphism. Ecol Lett 14:295-300.

560 Franks, D. W. and G. S. Oxford. 2017. The co-evolution of anti-predator
561   polymorphisms in sympatric populations. Biol J Linn Soc 122:729-737.

562 Gautier, M., J. Yamaguchi, J. Foucaud, A. Loiseau, A. Ausset, B. Facon, B.
563   Gschloessl, J. Lagnel, E. Loire, H. Parrinello, D. Severac, C. Lopez-Roques,
564   C. Donnadieu, M. Manno, H. Berges, K. Gharbi, L. Lawson-Handley, L.-S.
565   Zang, H. Vogel, A. Estoup, and B. Prud'homme. 2018. The genomic basis of
566   colour pattern polymorphism in the harlequin ladybird. bioRxiv.

567 Grindon, A. J. and A. Davison. 2013. Irish *Cepaea nemoralis* land snails have a
568   cryptic Franco-Iberian origin that is most easily explained by the movements
569   of Mesolithic humans. PLoS One 8:e65792.

570 Hart, N. S. 2001. Variations in cone photoreceptor abundance and the visual ecology
571   of birds. Journal of comparative physiology. A, Sensory, neural, and
572   behavioral physiology 187:685-697.

573 Hart, N. S., J. C. Partridge, I. C. Cuthill, and A. T. Bennett. 2000. Visual pigments, oil
574   droplets, ocular media and cone photoreceptor distribution in two species of
575   passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus
576   merula* L.). J Comp Physiol A 186:375-387.

23

577 Hoekstra, H. E. 2006. Genetics, development and evolution of adaptive pigmentation
578   in vertebrates. Heredity 97:222-234.

579 Holmes, I. A., M. R. Grundler, and A. R. D. Rabosky. 2017. Predator perspective
580   drives geographic variation in frequency-dependent polymorphism. Am Nat
581   190:E78-E93.

582 Hugall, A. F. and D. Stuart-Fox. 2012. Accelerated speciation in colour-polymorphic
583   birds. Nature 485:631-634.

584 Jones, J. S., B. H. Leith, and P. Rawlings. 1977. Polymorphism in *Cepaea*: a
585   problem with too many solutions? Annu Rev Ecol Syst 8:109-143.

586 Joron, M., L. Frezal, R. T. Jones, N. L. Chamberlain, S. F. Lee, C. R. Haag, A.
587   Whibley, M. Becuwe, S. W. Baxter, L. Ferguson, P. A. Wilkinson, C. Salazar,
588   C. Davidson, R. Clark, M. A. Quail, H. Beasley, R. Glithero, C. Lloyd, S. Sims,
589   M. C. Jones, J. Rogers, C. D. Jiggins, and R. H. ffrench-Constant. 2011.
590   Chromosomal rearrangements maintain a polymorphic supergene controlling
591   butterfly mimicry. Nature 477:203-206.

592 Kang, C., M. Stevens, J. Y. Moon, S. I. Lee, and P. G. Jablonski. 2015. Camouflage
593   through behavior in moths: the role of background matching and disruptive
594   coloration. Behav Ecol 26:45-54.

595 Kerkvliet, J., T. de Boer, M. Schilthuizen, and K. Kraaijeveld. 2017. Candidate genes
596   for shell colour polymorphism in *Cepaea nemoralis*. Peerj 5:e3715.

597 Kopp, M. and J. Hermisson. 2006. The evolution of genetic architecture under
598   frequency-dependent disruptive selection. Evolution 60:1537-1550.

599 Kunte, K., W. Zhang, A. Tenger-Trolander, D. H. Palmer, A. Martin, R. D. Reed, S.
600   P. Mullen, and M. R. Kronforst. 2014. Doublesex is a mimicry supergene.
601   Nature 507:229–232.

602 Li, J. H., J. M. Cocker, J. Wright, M. A. Webster, M. McMullan, S. Dyer, D.
603   Swarbreck, M. Caccamo, C. van Oosterhout, and P. M. Gilmartin. 2016.
604   Genetic architecture and evolution of the S locus supergene in *Primula*
605   *vulgaris*. Nat Plants 2.

606 Llaurens, V., A. Whibley, and M. Joron. 2017. Genetic architecture and balancing
607   selection: the life and death of differentiated variants. Mol Ecol 26:2430-2448.

608 Maia, R., C. M. Eliason, P. P. Bitton, S. M. Doucet, and M. D. Shawkey. 2013. pavo:
609   an R package for the analysis, visualization and organization of spectral data.
610   Methods Ecol Evol 4:906-913.

611 Mann, K. and D. J. Jackson. 2014. Characterization of the pigmented shell-forming
612   proteome of the common grove snail *Cepaea nemoralis*. BMC Genomics 15.

613 McKinnon, J. S. and M. E. R. Pierotti. 2010. Colour polymorphism and correlated
614   characters: genetic mechanisms and evolution. Mol Ecol 19:5101-5125.

615 McLean, C. A. and D. Stuart-Fox. 2014. Geographic variation in animal colour
616   polymorphisms and its role in speciation. Biol Rev (Camb) 89:860-873.

617 Montgomerie, R. 2006. Analysing Colors. Pp. 90-147 *in* G. E. Hill, and K. J. McGraw,
618   eds. Bird Coloration. Cambridge (MA): Harvard University Press.

619 Osorio, D., A. Miklosi, and Z. Gonda. 1999. Visual ecology and perception of
620   coloration patterns by domestic chicks. Evol Ecol 13:673-689.

621 Osorio, D. and M. Vorobyev. 2005. Photoreceptor spectral sensitivities in terrestrial
622   animals: adaptations for luminance and colour vision. Proceedings of the
623   Royal Society B-Biological Sciences 272:1745-1752.

624 Ozgo, M., T. S. Liew, N. B. Webster, and M. Schilthuizen. 2017. Inferring
625   microevolution from museum collections and resampling: lessons learned
626   from *Cepaea*. Peerj 5.

627 Ożgo, M. and M. Schilthuizen. 2012. Evolutionary change in *Cepaea nemoralis* shell
628   colour over 43 years. Global Change Biology 18:74-81.

629 Paterson, J. E. and G. Blouin-Demers. 2017. Distinguishing discrete polymorphism
630   from continuous variation in throat colour of tree lizards, *Urosaurus ornatus*.
631   Biol J Linn Soc 121:72-81.

632 Richards, P. M., M. M. Liu, N. Lowe, J. W. Davey, M. L. Blaxter, and A. Davison.
633   2013. RAD-Seq derived markers flank the shell colour and banding loci of the
634   *Cepaea nemoralis* supergene. Mol Ecol 22:3077-3089.

635 San-Jose, L. M. and A. Roulin. 2017. Genomics of coloration in natural animal
636   populations. Philos Trans R Soc Lond B Biol Sci 372.

637 Schilthuizen, M. 2013. Rapid, habitat-related evolution of land snail colour morphs on
638   reclaimed land. Heredity 110:247-252.

639 Scrucca, L., M. Fop, T. B. Murphy, and A. E. Raftery. 2016. Mclust 5: clustering,
640   classification and density estimation using Gaussian finite mixture models.
641   The R Journal 8:205-233.

642 Silvertown, J., L. Cook, R. Cameron, M. Dodd, K. McConway, J. Worthington, P.
643   Skelton, C. Anton, O. Bossdorf, B. Baur, M. Schilthuizen, B. Fontaine, H.
644   Sattmann, G. Bertorelle, M. Correia, C. Oliveira, B. Pokryszko, M. Ozgo, A.
645   Stalazas, E. Gill, U. Rammul, P. Solymos, Z. Feher, and X. Juan. 2011.
646   Citizen science reveals unexpected continental scale evolutionary change in a
647   model organism. Plos One 6:e18927.

648 Sinervo, B. and C. M. Lively. 1996. The rock–paper–scissors game and the evolution
649   of alternative male strategies. Nature 380:240.

650 Spottiswoode, C. N. and M. Stevens. 2010. Visual modeling shows that avian host
651   parents use multiple visual cues in rejecting parasitic eggs. Proc Natl Acad
652   Sci USA 107:8672-8676.

25

653 Spottiswoode, C. N. and M. Stevens. 2011. How to evade a coevolving brood
654   parasite: egg discrimination versus egg variability as host defences. Proc R
655   Soc Biol Sci Ser B 278:3566-3573.

656 Staples-Browne, R. 1908. On the inheritance of colour in domestic pigeons, with
657   special reference to reversion. Proc Zool Soc Lond 78:67-104.

658 Surmacki, A., A. Ozarowska-Nowicka, and Z. M. Rosin. 2013. Color polymorphism in
659   a land snail *Cepaea nemoralis* (Pulmonata: Helicidae) as viewed by potential
660   avian predators. Naturwissenschaften 100:533-540.

661 Taylor, C. H., T. Reader, and F. Gilbert. 2016. Hoverflies are imperfect mimics of
662   wasp colouration. Evol Ecol 30:567-581.

663 Teasdale, L. C., M. Stevens, and D. Stuart-Fox. 2013. Discrete colour polymorphism
664   in the tawny dragon lizard (*Ctenophorus decresii*) and differences in signal
665   conspicuousness among morphs. J Evol Biol 26:1035-1046.

666 Thompson, M. J. and C. D. Jiggins. 2014. Supergenes and their role in evolution.
667   Heredity 113:1-8.

668 Tripathi, N., M. Hoffmann, E. M. Willing, C. Lanz, D. Weigel, and C. Dreyer. 2009.
669   Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait
670   loci analysis of male size and colour variation. Proc R Soc Biol Sci Ser B
671   276:2195-2208.

672 van't Hof, A. E., P. Campagne, D. J. Rigden, C. J. Yung, J. Lingley, M. A. Quail, N.
673   Hall, A. C. Darby, and I. J. Saccheri. 2016. The industrial melanism mutation
674   in British peppered moths is a transposable element. Nature 534:102-105.

675 Vercken, E., B. Sinervo, and J. Clobert. 2008. Colour variation in female common
676   lizards: why we should speak of morphs, a reply to Cote et al. J Evol Biol
677   21:1160-1164.

678 Vorobyev, M. and D. Osorio. 1998. Receptor noise as a determinant of colour
679   thresholds. Proc R Soc Lond B Biol Sci 265:351-358.

680 Vorobyev, M., D. Osorio, A. T. D. Bennett, N. J. Marshall, and I. C. Cuthill. 1998.
681   Tetrachromacy, oil droplets and bird plumage colours. J Comp Physiol A
682   183:621-633.

683 Wellenreuther, M., E. I. Svensson, and B. Hansson. 2014. Sexual selection and
684   genetic colour polymorphisms in animals. Mol Ecol 23:5398-5414.

685 Wheldale, M. 1907. The inheritance of flower colour in *Antirrhinum majus*. Proc R
686   Soc Biol Sci Ser B 79:288-305.

687 White, T. E. and D. J. Kemp. 2016. Color polymorphic lures target different visual
688   channels in prey. Evolution 70:1398-1408.

689 Williams, S. T. 2017. Molluscan shell colour. Biol Rev (Camb) 92:1039–1058.

690

Table 1. Comparison between human perceived colour categories and Mclust-

defined groups. Shells that were scored the same are on the diagonal (in bold).

Yellow and pink were most common, and so the absolute number of discordant

scores was relatively low. Brown had by far the highest proportion of discordant

scores.

		Colour (human)				
		yellow	pink	brown	total	% total misclassified
Colour	yellow	**495**	44	0	539	8.2
(Mclust)	pink	118	**379**	14	511	25.8
	brown	9	90	**23**	122	81.1
	total	622	513	37		

Table 2. Results of likelihood ratio tests of the terms in binomial GLMMs of the effects of geographic variables and banding phenotype on the probability that a snail belonged to each of the three colour morphs. Significant p-values are in bold. The effects of modelling large-scale geographic variation in two ways are illustrated by the AIC values for the best model in which linear and quadratic effects of latitude and longitude were included (AICa), and the best model in which geographic region was included (AICb). All models include a random effect for site.

	Brown			**Pink**			**Yellow**		
AICa (df)				865.2 (11)			850.2 (8)		
AICb (df)				875.4 (22)			872.09 (22)		
Term	df	χ2	p	df	χ2	p	df	χ2	p
Latitude	1	1.747	0.186	1	0.218	0.641	1	2.62	0.106
Longitude	1	1.572	0.21	1	1.382	0.24	1	0.001	0.986
Latitude^2	1	2.019	0.155	1	1.832	**< 0.001**	1	4.732	**0.03**
Longitude^2	1	2.587	0.108	1	0.214	0.644	1	0.355	0.551
Banding	2	10.751	**0.005**	2	0.286	0.867	2	3.056	0.217
Latitude x longitude	1	0.239	0.625	1	0.0877	0.767	1	0.023	0.88
Latitude x banding	2	1.411	0.494	2	6.345	**0.042**	2	4.972	0.083
Longitude x banding	2	4.255	0.119	2	12.935	**0.002**	2	27.043	**< 0.001**
Region	6	10.586	0.102	6	7.481	0.279	6	5.924	0.432
Banding x region	12	12.824	0.382	12	34.564	**0.001**	12	31.448	**0.002**

Figure 1. Sample sites across Europe, grouped by geographically contiguous regions. A (England, n=397), B (Ireland, n=144), C (North Spain and Pyrenees, n=112), D (North France, Belgium, Germany, n= 178), E (Scandinavia, n=77), F (Poland, n=126) and all others (n=138).
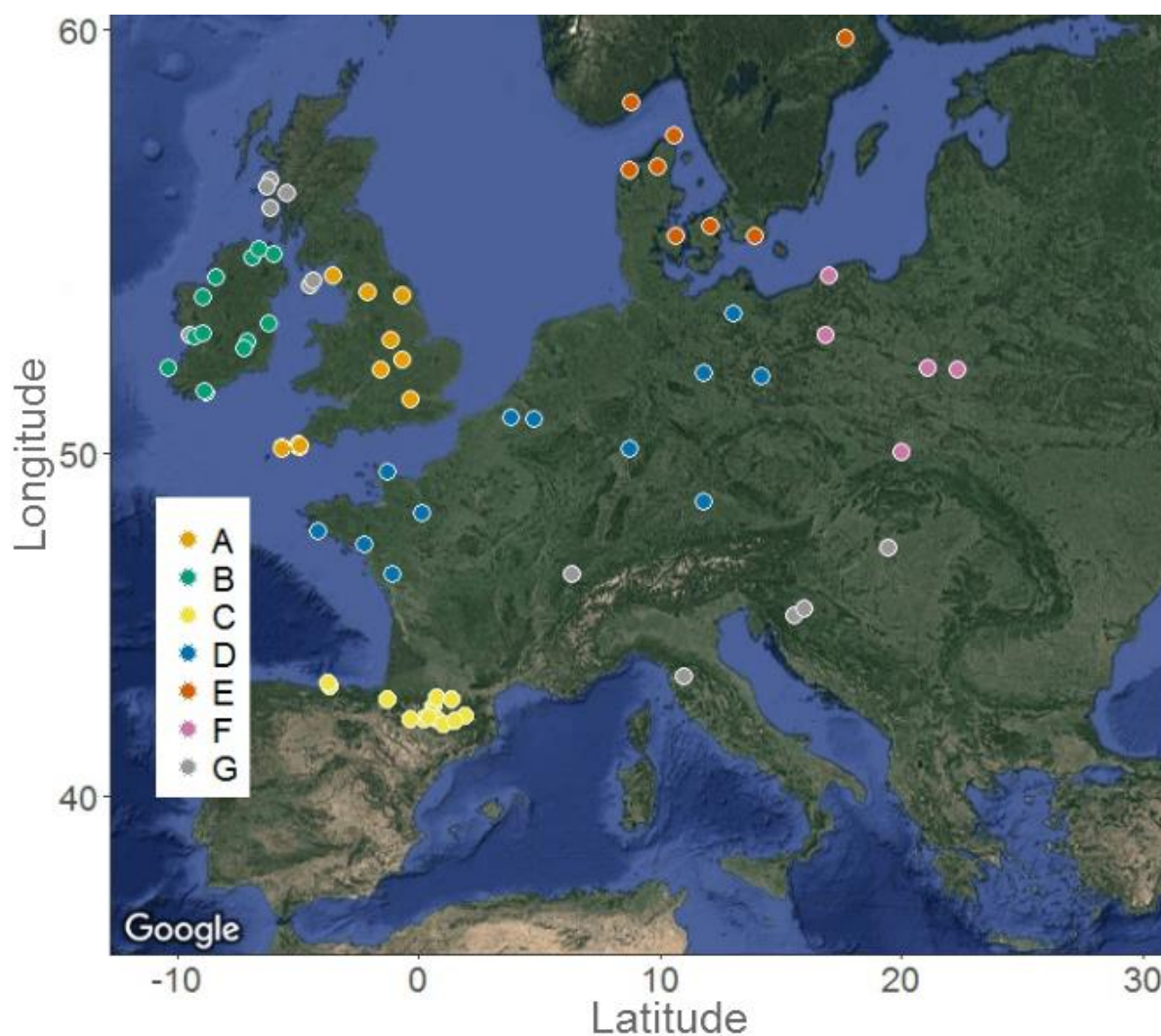
Figure 2. Axes of chromatic variation in the shell of *C. nemoralis*, using avian visual space, shown from two different perspectives (see also Supplementary Movie 2) . Units on x, y and z axes are in JNDs. The solid lines illustrate variation along the first three principal components; individual points are coloured according to Mclust classification of the shell, either yellow, pink or brown. Top: Variation along PC1 (87%) mainly represents differences in saturation between shells. PC2 (11%) shows relatively higher stimulation of L cones and lesser stimulation of M and S cones, and tends to separate brown from pink/yellow. Bottom: PC3 (2%) shows relatively high stimulation of the M cones compared to lesser stimulation of the S and L cones, and tends to separate yellow from pink and pink from brown.
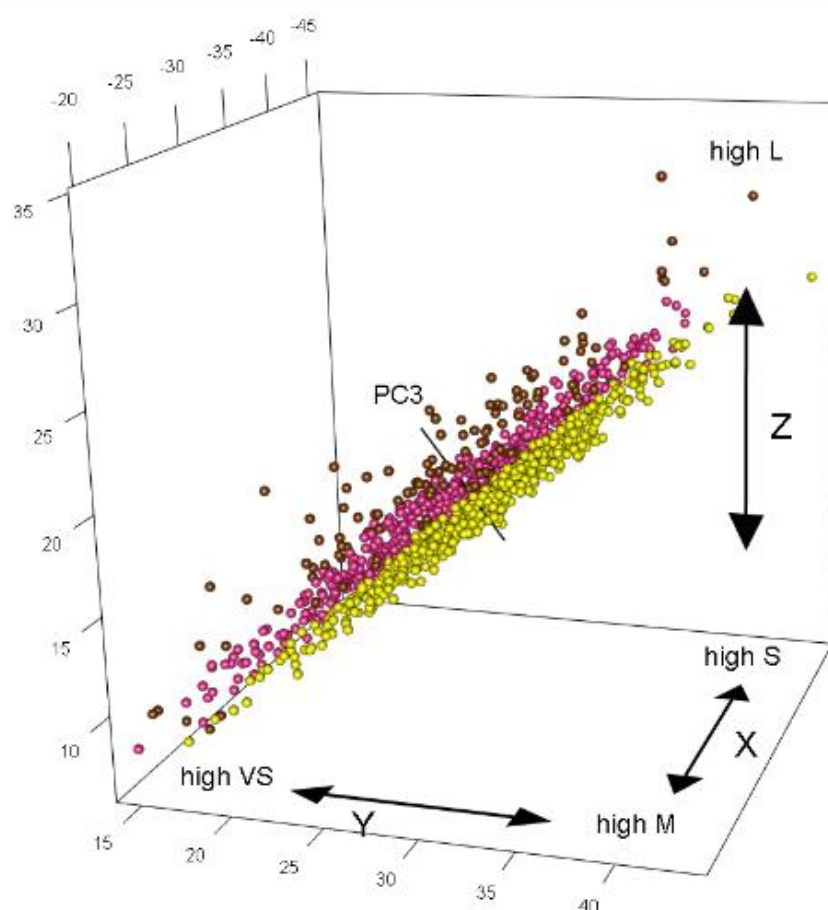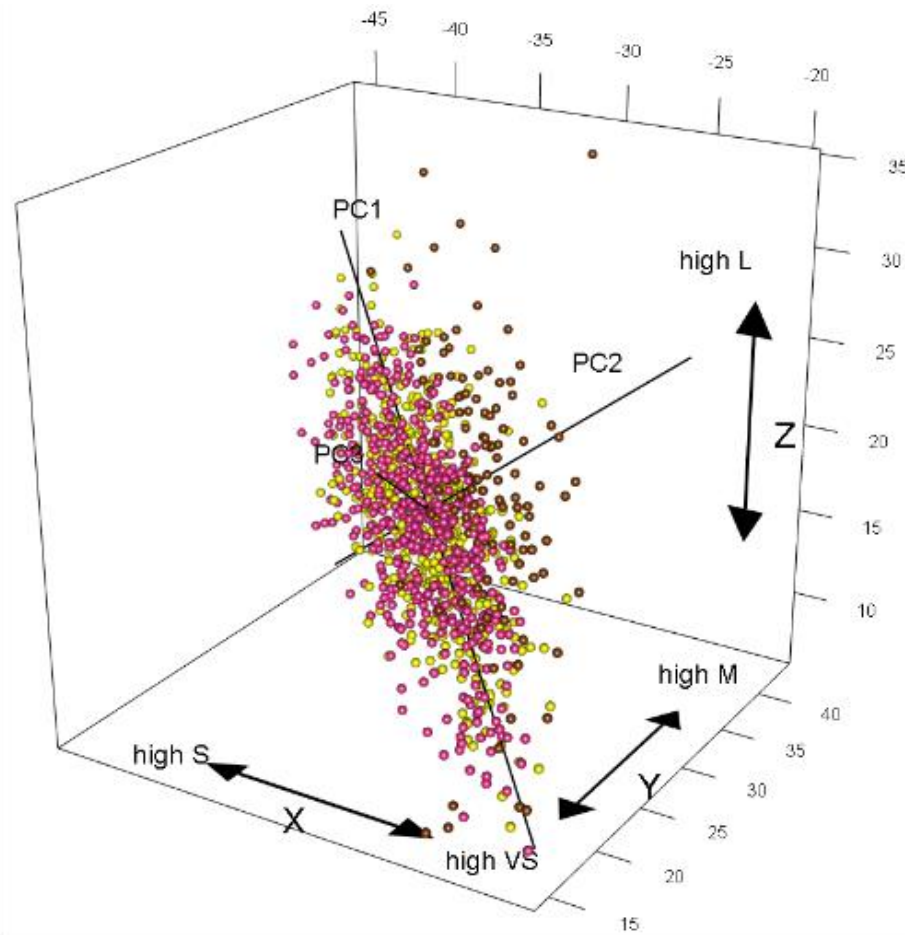
Figure 3. Interquartile ranges of the average normalised reflectance spectra for the principal component axes shown in Figure 2. These plots confirm that variation on PC1 mainly represents differences in saturation between shells; PC2 represents relatively higher stimulation of L cones and lesser stimulation of M and S cones; PC3 represents relatively high stimulation of the M cones compared to lesser stimulation of the S and L cones.
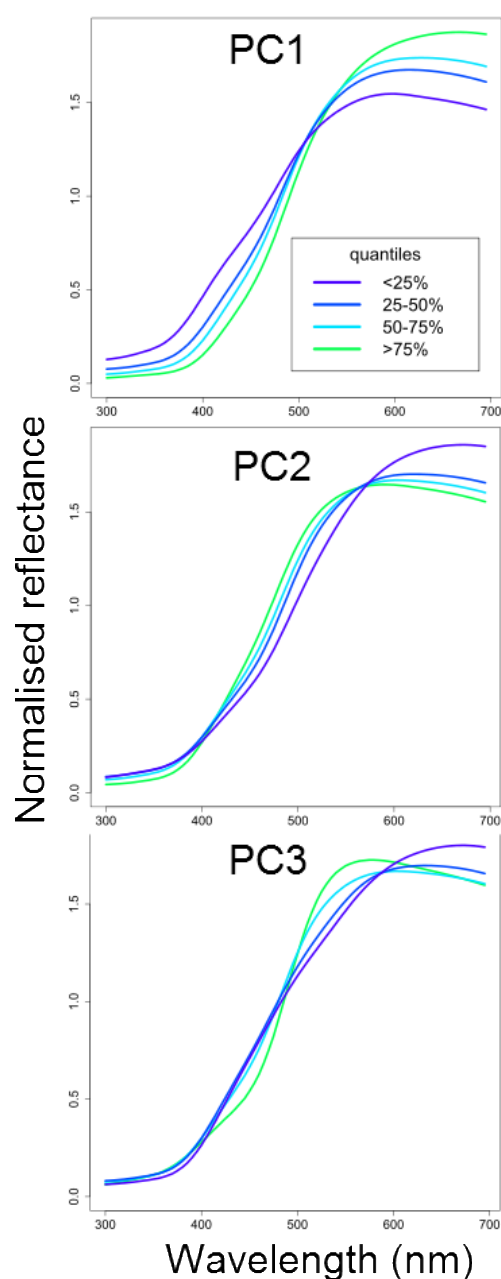
Figure 4. Scatterplot and associated density plot, showing variation along three principal component axes. Units are in JNDs. Points are coloured according to Mclust classification of the shell, either yellow, pink or brown. Top: PC1 versus PC2. Bottom: PC2 versus PC3.
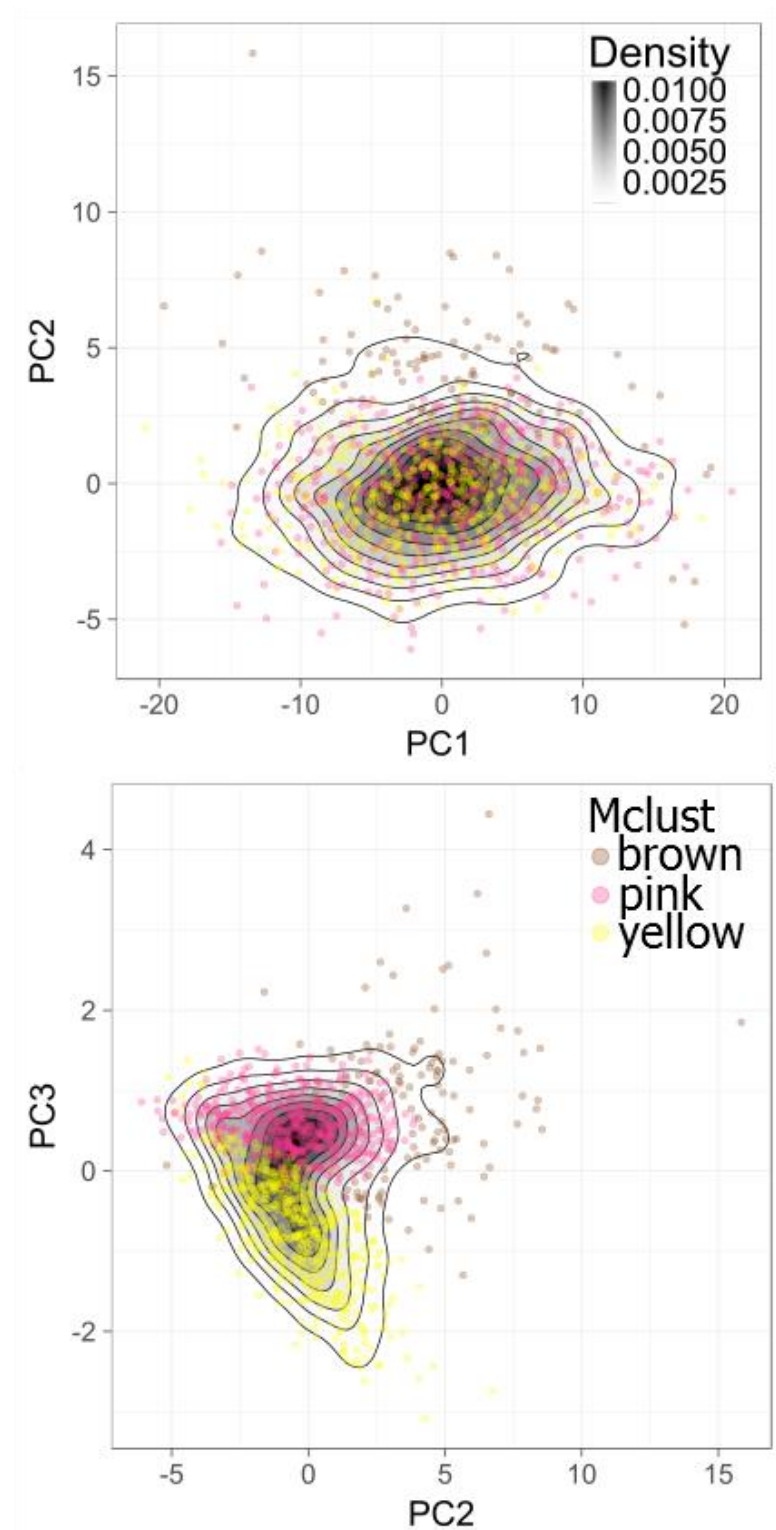
Figure 5. Scaled effects of latitude on the proportion of pink shells (top), and longitude on banding and proportion of pink shells.
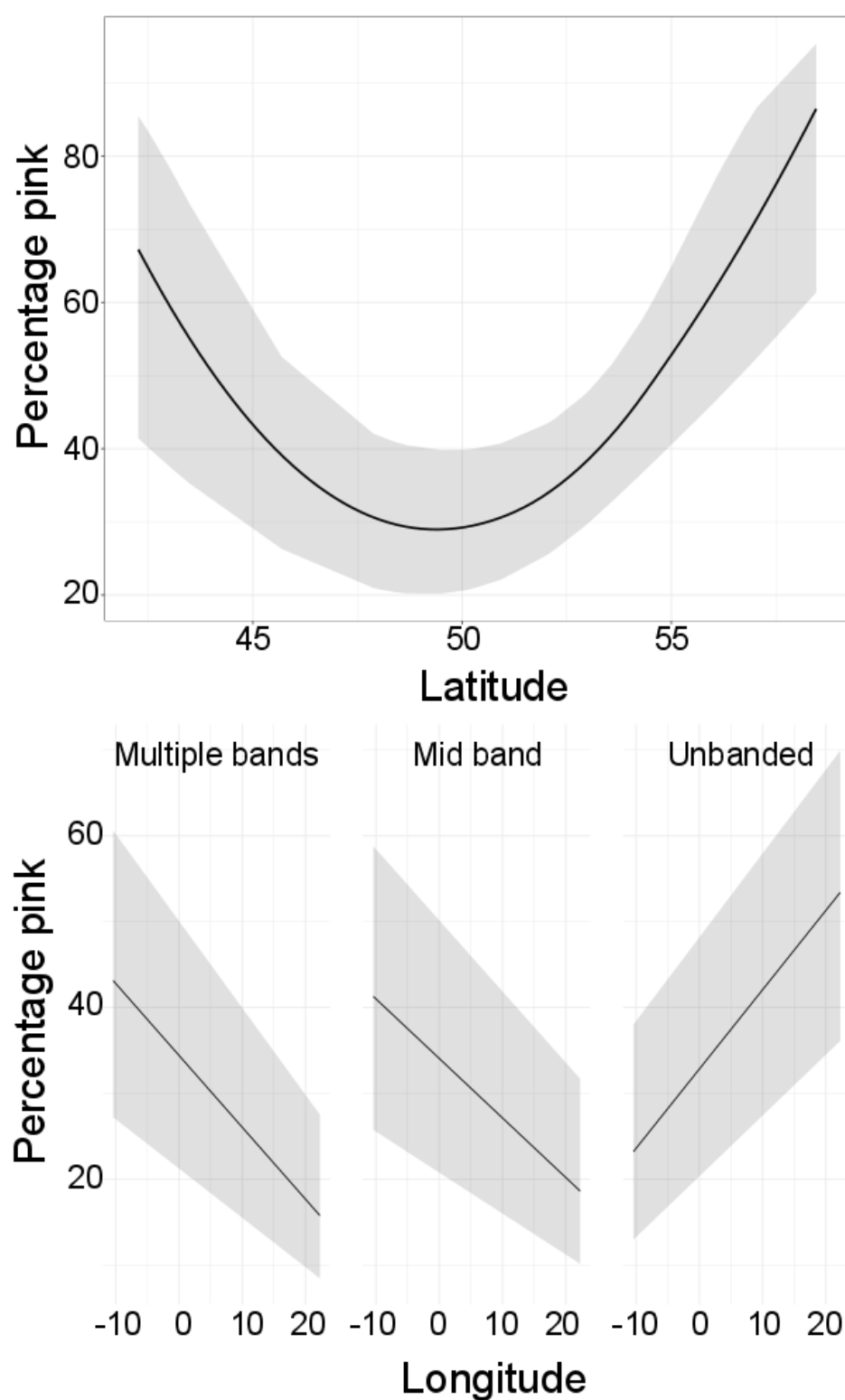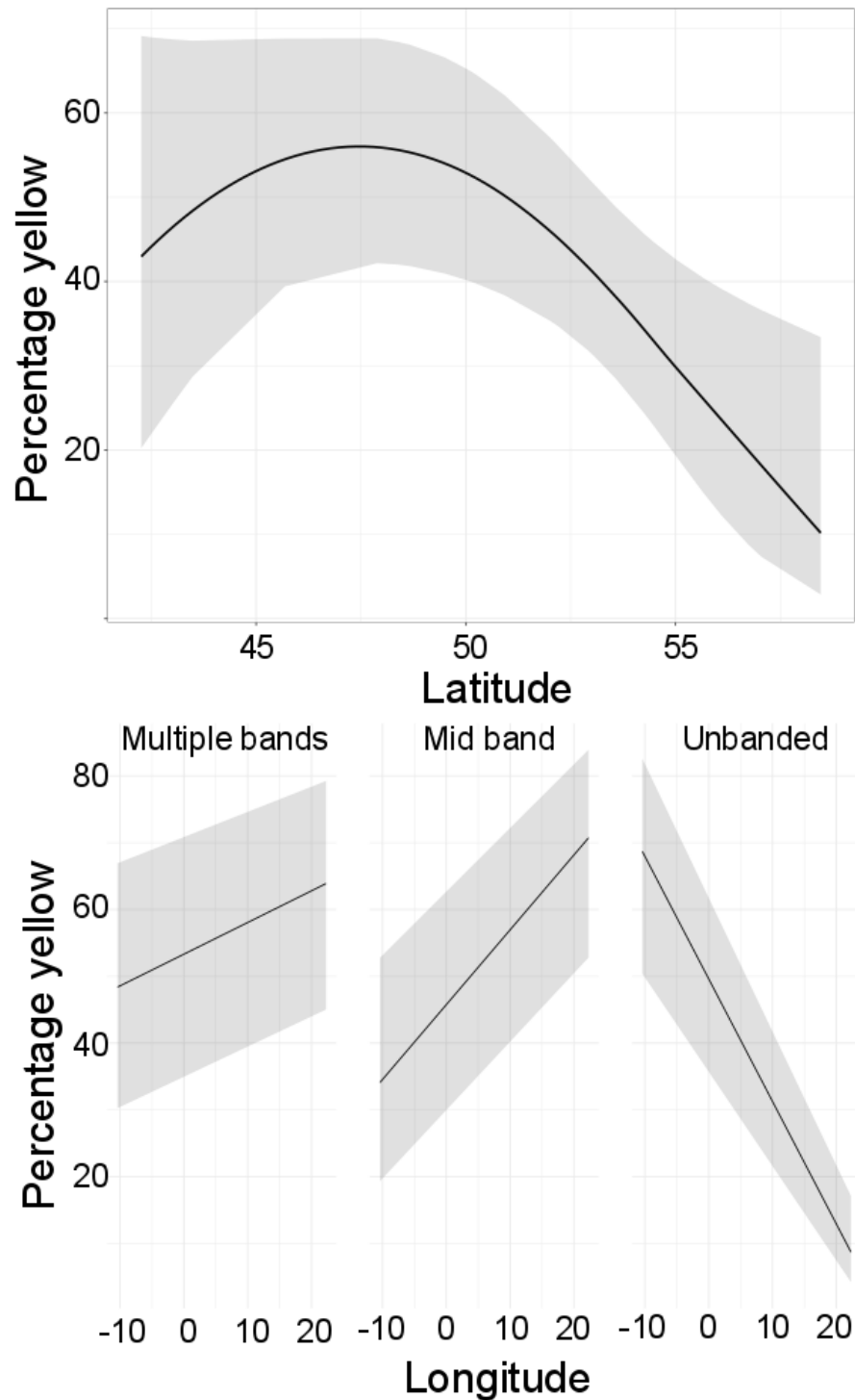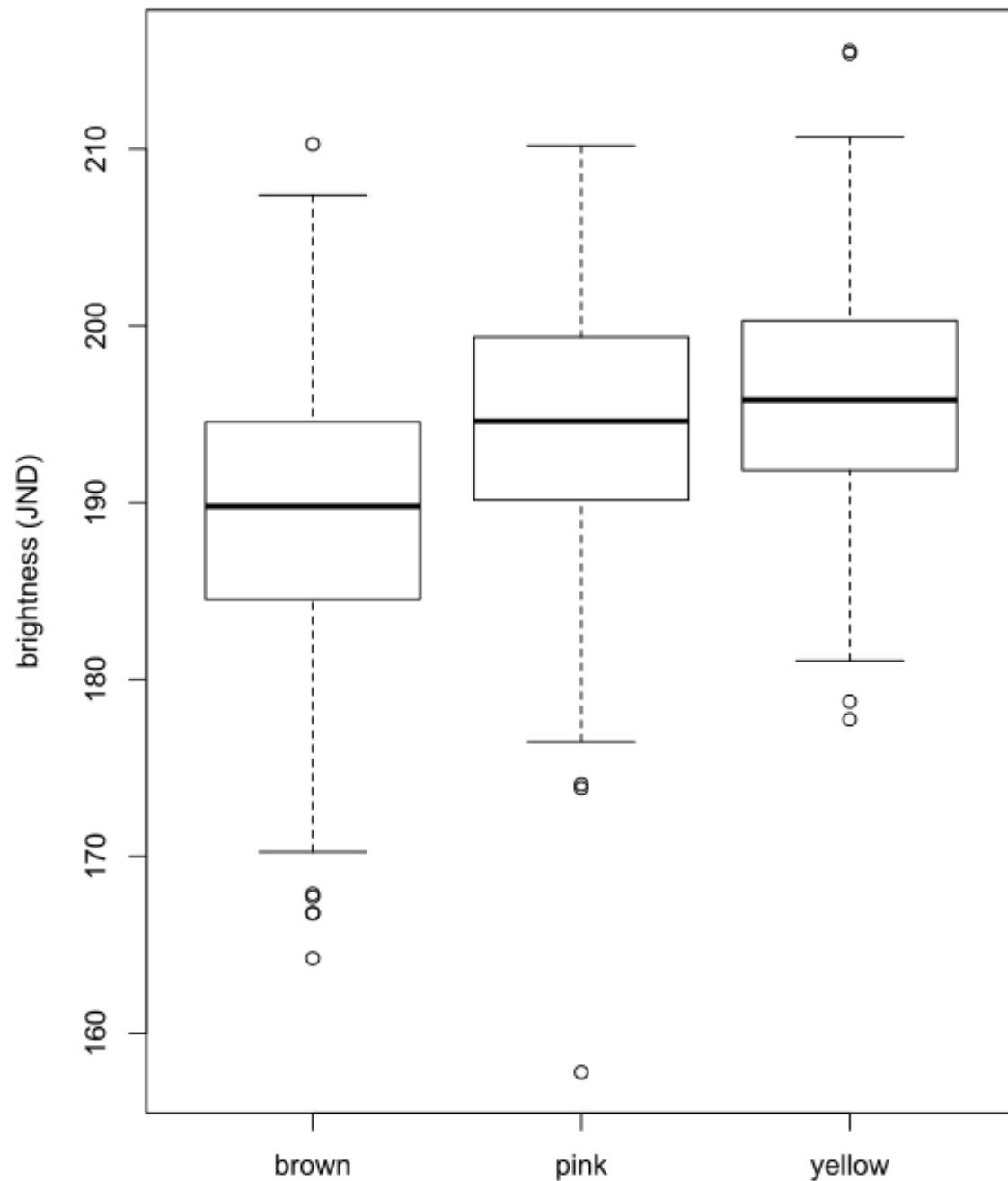
Figure 6. Scaled effects of latitude on the proportion of yellow shells (top), and

longitude on banding and proportion of yellow shells.

Supplementary Figure 1. Boxplot showing extent of achromatic variation in Mclust-defined colour morphs of *Cepaea nemoralis*.

Supplementary Movie 1. Animation showing axes of chromatic variation in the shell of *C. nemoralis*, using avian visual space. Units on x, y and z axes are in JNDs. The solid lines illustrate variation along the first three principal components; individual points are coloured according to human-scoring of the shell, either yellow, pink or brown.

Supplementary Movie 2. Animation showing axes of chromatic variation in the shell of *C. nemoralis*, using avian visual space. Units on x, y and z axes are in JNDs. The solid lines illustrate variation along the first three principal components; individual points are coloured according to Mclust classification of the shell, either yellow, pink or brown.