

1 **Report**

2 **Commercial harvesting has driven the evolution**
3 **of camouflage in an alpine plant**

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16 **Summary**

17 Colour in nature mediates numerous among and within species interactions [1], and
18 anthropogenic impacts have long had major influences on the colour evolution of
19 wild animals [2]. An under-explored area is commercial harvesting, which in animals
20 can exert a strong selection pressure on various traits, sometimes greater even than
21 natural selection or other human activities [3,4]. Natural populations of plants that
22 are used by humans have likely also suffered strong pressure from harvesting, yet the
23 potential for evolutionary change induced by humans has received surprisingly little
24 attention [5]. Here we show that the leaf coloration of a herb used in traditional
25 Chinese medicine (*Fritillaria delavayi*) varies among populations, with leaves
26 matching their local backgrounds most closely. The degree of background matching
27 correlates with estimates of harvest pressure, with plants being more cryptic in
28 heavily collected populations. In a human search experiment, the time it took
29 participants to find plants was greatly influenced by target concealment. These
30 results point to humans as driving the evolution of camouflage in populations of this
31 species through commercial harvesting, changing the phenotype of wild plants in an
32 unexpected and dramatic way.

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35 **Results and Discussion**

36 In the last decade, camouflage through background matching has been verified as a
37 defensive strategy in a number of plants, functioning to reduce herbivory [6-8], with
38 the degree of background matching linked to the level of selection pressure [9].
39 *Fritillaria delavayi* is a perennial herb distributed in the alpine scree from the
40 Hengduan mountains. It has leaves only at a young age and produces a single flower
41 per year after the fifth year. Adult plants flower in summer (June) and die away in
42 winter (October) annually. Leaf colour of *F. delavayi* varies among populations from
43 grey to brown, to green (Figure 1A-D). Grey or brown types appear well camouflaged,
44 while green individuals are conspicuous. Yet, after investigating all the accessible
45 populations in NW Yunnan in the past five years, we found few herbivory marks on *F.*

46 *delavayi*, and cannot identify any natural enemies of these plants. We are also
47 unaware of any study reporting herbivory. However, the bulb of this plant (“Lu Bei”)
48 is an important source of Chinese traditional medicine “Chuan Bei Mu”. These wild
49 herbs have been used for more than 2,000 years. The price of *F. delavayi* bulbs has
50 increased in recent years, reaching 3,200 CNY (ca. 480 USD) per kilogram (Data S1).
51 The mean dry weight of a single bulb is ca. 0.28 g, and over 3,500 individuals are
52 required to harvest just 1 kg of bulbs. Thus, harvest pressure on *Fritillaria* is high.

53 Colour divergence and local adaptation for camouflage provide evidence for
54 differential selection among populations. We measured leaf and rock colours in eight
55 populations from SW China (locations and sample sizes are shown in Table S1). We
56 found significant colour divergence among populations in CIE $L^*a^*b^*$ colour space, a
57 widely used vision model designed for human colour and luminance (lightness) vision
58 (Figure S1, MANOVA on coordinates L^* , a^* and b^* , *Pillai's trace* = 1.76, $F = 29.60$, $P <$
59 0.001). Leaf differences among populations on colour alone are significant (Figure
60 1A, MANOVA on coordinates a^* and b^* , *Pillai's trace* = 1.54, $F_{7,147} = 69.53$, $P < 0.001$),
61 with much greater divergence in camouflaged populations (ML, PY and PJ) than for
62 green populations (SK, TB and YL). Divergence was also found for luminance alone,
63 but the effects are not as strong as those of colour (Figure S2A). Populations ML, PY,
64 and PJ are well camouflaged in either the chromatic (a^* and b^*) or the luminance (L^*)
65 dimensions. In these populations, leaf colours matched their native rock backgrounds
66 better than they matched alien backgrounds (Figure S2, one-way ANOVAs, $P < 0.01$),
67 showing that the current colour divergence of *Fritillaria delavayi* is not random, but a
68 result of population-specific selection.

69 We investigated the association between background matching (based on colour
70 distances between leaves and local backgrounds) and potential harvest pressure
71 intensity. Harvest pressure was estimated with two measures: collection intensity
72 and collection difficulty. To estimate collection intensity experienced by a population,
73 the total collected amount of *F. delavayi* (dry weight from 2014 to 2019, Data S1)
74 reported for each population was divided by the relative abundance (mean plant
75 number in plot) of *F. delavayi* in the corresponding population. We found a

76 significant negative relationship between colour distance and collection intensity
77 (Figure 2B, collection intensity was sqrt-transformed, Spearman *rho* correlation, $r = -$
78 0.836 , $df = 137$, $P < 0.001$), indicating that plants are better camouflaged in
79 populations with heavier harvesting.

80 Collection difficulty was estimated by the time (in seconds) spent digging out a
81 single bulb using a tool, which depends mainly on the bulb depth and the rocky
82 substrate structure, with both factors varying among populations. Bulbs deep under
83 tightly stacked big rocks take longer to dig out, and are less heavily collected. We
84 found a significant positive relationship between colour distance and collection
85 difficulty (Spearman *rho* correlation, $r = 0.678$, $df = 138$, $P < 0.001$). As predicted,
86 plants in populations that are easier to collect are better camouflaged (Figure 2C). An
87 exception is population LJ (Yulong Mt., Lijiang), where the collection is not too
88 difficult (mean collection time 37.8s) but the plant is green. This exception can be
89 explained by the low collection intensity in this population.

90 To test the prediction that improved match to the background results in longer
91 detection times, we developed an online citizen science experiment “spot the
92 *Fritillaria*” (www.plant.sensoryecology.com). Humans have long been used to test
93 questions related to target salience using visual displays (e.g. [10]), and are widely
94 used in recent computer-based experiments to test camouflage concepts with more
95 naturalistic stimuli (e.g. [11]). Human subjects were asked to locate a fritillary target
96 as quickly as possible in each of 14 randomly allocated photo slides, simulating the
97 herb collection process by collectors. They had a free choice to play as either a
98 trichromatic or dichromatic condition, which used images with three (red, green and
99 blue) or two (blue-yellow) colour channels, respectively. As humans are trichromats
100 and most other mammalian herbivores are dichromats, this set up allowed us to
101 compare the search efficiency between human and potential natural herbivores. As
102 expected, targets with lower salience values (better camouflage) required longer
103 times to be located (Figure 2D, Table S1). Trichromatic players spent less time
104 locating targets than the simulated dichromatic players (3.99 ± 0.04 vs 3.29 ± 0.06 s,
105 mean \pm se.). Given the intense commercial harvest of this species, these results show

106 that the visual phenotype of *F. delavayi* may greatly influence its fitness.
107 Furthermore, humans, being predominantly trichromatic, can exert a stronger
108 pressure than other potential dichromatic mammalian herbivores (if any exist) on
109 colour evolution.

110 In principle, plant camouflage could be a result of natural selection by wild
111 herbivores, which could have been more common in the past when the frequency of
112 human activity was low. However, herbivores are currently very rare in the area we
113 studied. We have not observed any animal (including free-ranging yaks, the large
114 domestic herbivores) that feeds on either the leaf or the bulb of *F. delavayi* in any of
115 the eight populations. In fact, *Fritillaria* species are rich in alkaloid chemical defences,
116 which are known to be effective in deterring herbivores, such as rodents [12].
117 Ironically, it is such alkaloid compounds that have made it a medicinal herb and
118 induced collection. More importantly, other potential natural enemies seem unlikely
119 to have driven the present correlation between background matching and measures
120 of harvest intensity.

121 In animals, selective hunting by humans is reported to result in smaller weapon
122 (e.g. horns or antlers) size in ungulates, but such an effect has been suggested to be
123 limited because hunted males often reproduce before they are shot [13]. Both young
124 and adult fritillary are harvested, with smaller (younger) bulbs being sold for higher
125 prices in the retail market. Our results show that the colour of *Fritillaria delavayi*
126 varies among populations and closely matches their local background, with the
127 degree of such background matching closely following the local harvest pressure for
128 this highly valued herb in Chinese traditional medicine. Our findings are consistent
129 with harvest pressure as the selective force driving colour evolution in this plant. To
130 further confirm this, more efforts are needed to rule out potential natural
131 herbivores. On the other hand, experimental approaches to quantify the fitness and
132 evolution of plant colour under a relaxed harvesting pressure would be valuable in
133 the long term. The efficacy of camouflage in these plants may also be affected by the
134 complexity of the visual environment, as this is known to strongly affect detection of
135 concealed targets (e.g. [14, 15]) and even the salience of flower signals to bees [16].

136 This and similar factors would be valuable to explore further. In the present study,
137 the fritillary collectors do not aim to select for colour directly, but their harvest
138 activity has influenced the adaptive evolution of plant colour, intensifying phenotypic
139 divergence. An analogue is Vavilovian mimicry (weed mimicry), where the
140 resemblance of weeds to crops has been regarded as a result of unintentional
141 selection by humans [17]. Given that humans have long collected animals and plants
142 for a variety of traits, we expect there to be many other analogous examples of
143 humans driving changes in coloration in the wild.

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161

162 **Author contributions:** All authors conceived and designed the study and
163 experiments. H.S. designed the strategies for plant sample collection and harvest
164 pressure estimation. M.S. designed the strategies for colour analyses and harvest
165 pressure estimation, and N.Y. and M.S. the computer experiment. N.Y. and M.S.

166 processed data and analysed results. N.Y., and H.S. prepared display items. Y.N., H.S.
167 and M.S. wrote the manuscript.

168

169 **Declaration of interests:** The authors declare no competing interests.

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171

172 **Figure 1 Plant colour variation of *Fritillaria delavayi* among populations.**

173 (A & B) Normal green individuals in populations with low harvest pressure. (C & D)

174 Camouflaged individuals in populations with high harvest pressure.

175

176

177 **Figure 2 Plant colour variation of *Fritillaria delavayi* among populations, and its**
178 **correlation with collection intensity, collection difficulty, and human search time.**
179 (A) Colour divergence from eight populations in human CIE L*a*b* colour space (see
180 also Figures S1 and S2). (B) Correlation between plant camouflage (match to the
181 background) and collection intensity (harvest amount divided by plant abundance).
182 (C) Correlation between plant camouflage and estimates of collection difficulty (time
183 spent collecting a single bulb, in seconds). (D) Capture time (milliseconds) decreasing
184 with increasing salience value (an image-based measure of how much a target
185 deviates from the background, without unit) in online visual selection experiment.
186 Note trichromats spend less time than simulated dichromats finding plants (See also
187 Table S1).
188

189 **STAR★METHODS**

190 **RESOURCE AVAILABILITY**

191 **Lead Contact**

192 Further information and requests for resources should be directed to and will be
193 fulfilled by the Lead Contact, Martin Stevens (martin.stevens@exeter.ac.uk).

194

195 **Materials Availability**

196 This study did not generate unique reagents.

197

198 **Data and code availability**

199 Source data for all the figures in the paper is available as a supplementary file (Data
200 S1).

201 The online visual science game is available at www.plant.sensoryecology.com

202

203 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

204 All human subjects ($N = 542$) that played the online game as ‘plant hunter’ and
205 performed the visual search task were informed the intention of the game before
206 they play. The gender of subjects is not collected, as this is not relevant to the aim of
207 our study. All subjects gave consent to take part in the trials, and for their data to be
208 used, and were free to leave the experiment at any time. This work was conducted
209 with the approval of the University of Exeter Biosciences ethical committee (No.
210 eCORN000353 v2.0). For colour analysis, only leaves were collected for
211 measurement. For collection difficulty estimation, we did not take the bulbs out, but
212 only recorded the time spent collecting, and then filled all the substrate (rocks) back.

213

214 **Methods Details**

215 **Materials and study locations**

216 *Fritillaria delavayi* Franch. (Liliaceae) is a perennial herb that is distributed in the
217 Himalaya-Hengduan mountains. The habitat of this plant is the alpine screes, bare or
218 sparsely vegetated, with elevation between 3700 to 5600 m a.s.l. [18]. Like most

219 plants in the alpine screes, *F. delavayi* grows from May to September, and its above-
220 ground parts die away when winter comes. Young individuals in the first one to three
221 years have only one leaf, with shape being short and needle-like (in the first year, less
222 than 1 cm wide, often folded), to ablong-ovate (in the second year, unfolded). In the
223 following years, it produces two or more leaves but does not flower. Plants often
224 flower after the fifth year, sometimes producing unisexual (female sterile) flowers in
225 the first flowering season. The adult plant usually has three to five leaves, and
226 produces only one flower and one bulb. The leaf colour of this plant varies within
227 and among populations. To human observers, plants from some populations seem to
228 match their substrate, and thus appear to be well camouflaged, whereas other
229 populations are perceived as green, and not matching their substrate. Interestingly,
230 flower colour also varies among populations. In the camouflaged populations, floral
231 colours also match the background very well.

232 Six *Fritillaria* taxa (five species, one with a variety) in China are listed as sources of
233 Chinese Traditional Medicine, collectively named “Chuan Bei Mu” [19]. Among them,
234 *F. delavayi* is the only species that grows in the alpine scree slopes at very high
235 elevation. It is recognized as “Lu Bei”, as a sub-category of “Chuan Bei Mu” in
236 pharmacopoeia and the market. These listed plants are of the most intensively
237 harvested medicinal herb in China [20], making China the biggest market for “Chuan
238 Bei Mu” and other related traditional medicine. Although not so famous as *F.*
239 *cirrrosa* (so called “Qing Bei” as a sub-category), bulbs of *F. delavayi* also have a high
240 price and are often fraudulently traded under the same name “Bulbus Fritillariae
241 Cirrhosae” [21].

242 Our study was conducted in eight locations (Table S1) in NW Yunnan and SW
243 Sichuan provinces, ranging in elevation from 3700 to 4800 m a.s.l. These plants are
244 exposed to strong temperature fluctuations and high UV-radiation. The screes in
245 these locations are formed by different rock types, either limestone or shale that
246 vary in colour.

247

248 **Colour measurements**

249 Colour was measured based on reflectance spectra from eight populations. Twelve to
250 27 leaf samples (specific sample sizes were shown in Table S1) from different
251 individuals were collected in the field for reflectance measurements. Flower colour
252 was not included in the analysis as a large proportion of plants were collected
253 without flowers, either because they were still of young age or because the harvest
254 season is often after flowering time. For the background, we collected 20 to 40 rocks
255 (specific sample sizes were shown in Table S1) within a radius of 5 cm of each focal
256 plant. All samples were kept in plastic bags and brought back to our guesthouse
257 (where AC power was available) within two hours and measured using spectrometer
258 FLAME equipped with HD2000 UV-VIS-NIR light source (OceanOptics, FL, USA). A
259 PTFE standard was used as a white standard. For both plants and rocks, the upper
260 surface of the sample, which was seen by the observers, was used in measurement.
261 Each sample was measured three times and then averaged before further analysis.
262 Reflectance between 390 and 750 nm was used in the calculation, as the expected
263 observers are human and other mammals.

264

265 ***Quantifying background matching***

266 Colour matching of plants against rock backgrounds was measured by colour
267 similarity between each target and rock, as measured by human vision models. To do
268 this, the spectrum of each sample was converted to photon catch values equivalent
269 to L, M and S cone responses [22], and mapped into CIE $L^*a^*b^*$ space, using equations
270 provided by international commission on illumination [23]. CIE $L^*a^*b^*$ is a scaled
271 opponent model that is specifically designed for human colour vision, with L^*
272 correlating to perceived lightness, a^* and b^* approximately describing green to red
273 and blue to yellow variations, respectively. It uses CIE XYZ tristimulus values as the
274 input data, which can be calculated from reflectance and irradiance spectra. It also
275 includes chromatic adaptation (“von Kries transformation”) to account for colour
276 constancy under different light conditions. Colour similarity was calculated as the
277 Euclidean distance between colour loci (the locations of the colour points) in a colour
278 space. A closer distance between plant and background colour loci indicates better

279 background matching. Luminance (perceived lightness) is indicated by L^* and was
280 analysed separately. Cone sensitivity curves (colour matching functions) were
281 obtained from <http://www.cvrl.org/cmfs.htm>. D65 (daylight) was used as the
282 irradiance condition. For the sake of comparison with other widely used metrics, we
283 also calculated colour distances in the form of Just Noticeable Differences (JNDs). The
284 Euclidean colour distance in CIE $L^*a^*b^*$ space is thought to be roughly equivalent to
285 JNDs, with 2.3 $L^*a^*b^*$ unit being approximately equal to 1 JND [24]. We calculated
286 colour differences using the updated CIEDE2000 colour-difference formula ([25], the
287 Excel spreadsheet implementation, available at
288 <http://www2.ece.rochester.edu/~gsharma/ciede2000/>), which uses CIE $L^*a^*b^*$ colour
289 coordinates as the input, with the output being JND colour differences to humans.
290 The results can be seen in Figure S2C, and are directly comparable to the results
291 obtained in CIE lab space (cf. Figure S2B).

292

293 **Estimation of harvest pressure**

294 An ideal way to estimate harvest pressure is to investigate the proportion of
295 harvested plants in each population over time for a long enough period that
296 evolution could act. However, such population-specific historical data cannot be
297 obtained. Therefore, we used two metrics that allowed us to estimate the harvest
298 pressure acting upon a population, the collection intensity and collection difficulty.
299 Collection intensity was calculated as:

$$300 \quad CI = (W_c / W_s) / (N \times S)$$

301 For each population, CI is the collection intensity (a ratio without unit), which is
302 represented by the number of collected individuals divided by the estimated number
303 of total individuals. W_c is the total dry weight (g) of plant bulbs collected in the most
304 recent six years (from 2014 to 2019). W_s is the mean dry weight (g) per single bulb
305 (individual), estimated from the bulbs purchased from these populations. N is the
306 mean number of plants per m^2 counted in the plots. S is the area (in m^2) that *F.*
307 *delavayi* distributed. Larger collection amounts (W_c / W_s) occurring in lower
308 abundance populations ($N \times S$) would yield higher collection intensity values. Dry

309 weights of bulbs collected (W_c) in the last six years were obtained from the local herb
310 dealer in the village close to the population we studied. There was only one head
311 herb dealer in the village, who gathered fritillary bulbs and other herbs from private
312 collectors. At least in the area we studied, each location (and the adjacent regions) is
313 managed, collected and utilized by a separate village to avoid any interest conflict.
314 The trading record of *F. delavayi* was written under the local plant name “Zhimu”.
315 Records from seven out of the eight populations were obtained. We could not obtain
316 data on the collection amount in population in ML (Saganai Mt., Muli, SW Sichuan
317 province), although recently dug collection potholes were seen. These data may
318 systemically underestimate the real collection amount, as the collectors may sell the
319 bulbs to tourists or use the bulbs for themselves as well. However, it should reflect a
320 general variation of collection among populations. The weight of a single bulb (W_s)
321 was measured from 100 to 200 bulbs in each population using a balance. To estimate
322 the abundance of *F. delavayi*, we counted the plant number in ten 2 m x 2 m plots in
323 about population and calculated the mean plant number per m^2 (N). The plots were
324 chosen in the very habitat that *F. delavayi* can be found. In an area about 300 m x
325 300 m, when we found an individual, the surrounding 2 m x 2 m area was set as a
326 plot, and checked carefully. We tried to count every individual in the plot, although
327 the very small needle-like first-year individuals in rock crevices could sometimes be
328 overlooked. Next, we estimated the distribution area (S) occupied by *F. delavayi* in
329 each population using high resolution satellite images accessed from GoogleEarth,
330 based on previous surveys in these regions. Specifically, we used the highest
331 resolution images in Google Earth Pro (V7.3.3.7786 , 2020CNES/Airbus), and used a
332 polygon tool to select the habitats carefully and obtained the area in m^2 . We did not
333 use an automatic approach to calculate the habitat area because it is not accurate
334 enough. The areas were selected based on the habitat of *F. delavayi* and accessibility.
335 Like other alpine scree plants, the distribution of *F. delavayi* greatly depends on the
336 microhabitat; i.e. it grows on screes composed of rocks that are not too large, and
337 never on meadows, shrubs and cliffs (which can be distinguished in the satellite
338 images).

339 Although collection intensity mentioned above reflects the harvest pressure at
340 least in the recent period, this intensity might have changed over a long history. As a
341 supplementary metric, we also estimated the collection difficulty, which may
342 significantly influence the intention of collection and harvest pressure. Despite their
343 commercial value, plant bulbs in alpine screes are often hard to collect. The
344 collection difficulty varies among populations, depending on the depth of the bulb
345 underground and the rock structure where the bulb grows, which varies among
346 populations. For example, bulbs deep under tightly stacked big rocks are very difficult
347 to collect. As far as we know, these factors *per se* do not influence the plant colour
348 phenotype. And importantly, this parameter remains unchanged through years.
349 Collection difficulty was measured as the time spent (in seconds) collecting a single
350 bulb by the authors, using a stop clock on a smart phone. It is possible that the
351 experienced local collectors may spend less time to dig out the bulbs, but our
352 estimate should reflect the general pattern of inter-population variation. Bulbs from
353 nine to 18 individuals were dug out to estimate the mean time spent for each
354 population, and then were backfilled. Seven populations were included in estimation
355 except population YG (Yagong Mt., Nixi), as we were prevented from revisiting this
356 location by debris flow.

357

358 **Visual selection assessments**

359 To simulate the selection process and test the prediction that improved match to the
360 background results in longer detection times, we developed an online citizen science
361 visual experiment based on photographs. A field survey in a collection area might be
362 a more straightforward approach, but we cannot control factors such as weather
363 (which may influence the background colour), light conditions, and the experience of
364 the collectors. More importantly, we cannot quantify the background matching of
365 collected plants without disturbing the collection process. Furthermore, to obtain
366 enough trials in the field, we would need to encourage collection, which is not our
367 intention for this plant, close to being endangered. In contrast, a visual online
368 experiment is powerful enough to obtain a general conclusion of this process, one

369 with which we can quantify the camouflage efficacy of each plant in the experiment
370 and obtain corresponding capture times. Similar experiments using online citizen
371 science games have proven to be a powerful way of assessing camouflage efficacy
372 and the role of colour vision in focal animals such as birds and crabs [11, 26].

373 **Photographs** Images of *Fritillaria* plants used in the game were taken from
374 several locations in Shangri-La (Tianbao, Shika, Hongshan) and Deqin (Pujin) NW
375 Yunnan province during June-July 2017. A Nikon D7100 with Tamron 90 mm lens
376 were used for taking photos at ca. 5 m away from the plant target on a non-raining
377 day. Aperture was kept at F/10. A photo always included both the target plants and
378 the surrounding rock backgrounds. To control for light conditions, a standard colour
379 checker (colour passport, X-rite) was set in a second photograph that shared the
380 same light conditions. Each photo only included a single focal plant, but other
381 accompanying plants may inevitably co-occur in some photos (as a distraction, which
382 was considered in the model). All plants were photographed from their visible
383 viewing angle to make sure they can be seen without obstacles. The location of the
384 target was made random, in different parts, but not on the very edge of the photo.
385 As both young and adult plants are harvested, photos included both of them. Photos
386 of various difficulty to locate (estimated by experience) were used in the game. In
387 camouflage search tasks, trichromats do not always perform better than dichromats.
388 Dichromatic vision has long been thought to have advantages in detecting
389 camouflage objects [27], therefore it is worth comparing it with trichromatic vision in
390 the visual experiment. To simulate the scene that viewed by a general mammalian
391 dichromat with long and shortwave cone types, all trichromatic photographs were
392 also converted into blue-yellow dichromatic images by combing red and green
393 channels ($Y = (R + G)/2$, in ImageJ, as used by Troscianko *et al.* [11]). A total of 48
394 photos were prepared in trichromatic and dichromatic versions each.

395 **Quantifying conspicuousness** We quantified the conspicuousness of plants in
396 each photo using a salience value [28]. This image-based parameter estimates target
397 camouflage (or more strictly, lack of camouflage) by taking both predator perception
398 and the visual background into account. It combines different visual attention-

399 relevant visual features, i.e., luminance, colour, and orientation contrasts, into a
400 single value to give holistic target conspicuousness taking into account the weight of
401 each feature. For the focal plant in each photo, the salience value was calculated
402 using a Matlab code modified from Pike [28] and Itti *et al.* [29], setting the weight of
403 colour, luminance and orientation to 1. For colour features, the code was modified to
404 describe three channel images.

405 **Games** We generated a free online game to work on internet browsers written
406 with JavaScript based on Troscianko *et al.*, [11] (www.plant.sensoryecology.com).
407 Subjects were expected to locate the fritillary target as soon as possible in each of
408 the 14 photo slides. A few plant samples were shown to inform the players of what
409 the target looked like and what the target did not look like. Subjects were asked
410 whether they had played this game before or not (experience), and whether they
411 would like to play as a human (trichromatic) or a yak (dichromatic). Then 14 random
412 (out of 48) slides of fritillaria photos were shown in a random order. The subjects had
413 up to 15 seconds to point out (by clicking the target as soon as they saw it) the
414 location of the target. Click coordinates were recorded. When the target region was
415 clicked successively, a green circle with a sound response was shown on the target
416 location, and the capture time was recorded (in milliseconds) and displayed, before
417 moving on to the next slide. Although various aspects of the technical set up of
418 online games could potentially limit the level of precision with regards to measuring
419 timing differences, most if not all of these would run at speeds substantially beyond
420 the reaction times recorded here; any possible limitations should be limited to within
421 or less than 10-20 ms. Even if such limitations exist, this would still afford high
422 precision with regards to human reactions, and any lag in the various technical
423 systems should be consistent across participants, and so could not explain the results
424 obtained. If the player failed to find the focal plant before the time was up, a red
425 circle was shown on the target location with a different sound, before moving on to
426 the next slide. An average capture time was shown after the game finished. All data
427 collected were anonymously, and no ID information was used to identify individuals.

428 Online experiments to test theories of camouflage and other forms of adaptive

429 coloration have become increasingly popular in recent years and proven to be a
430 powerful method of testing the efficacy of anti-predator defences (e.g. [11, 30]). Our
431 study is particularly well suited to this, given that the hypothesised observer is
432 humans themselves. Nonetheless, there are potential limitations with online
433 experiments [30], including that the monitors of participants are uncalibrated and
434 will vary. Nonetheless, we are confident that the approach is robust and accurate for
435 our key questions here. Most crucially, there is no reason to expect that display
436 variation would add any systematic bias in any direction, but instead should simply
437 add noise. In fact, the great advantage of online experiments is in the number of
438 participants that can be used, which work to greatly overcome any such noise
439 effects. The other potential issue, beyond colour reproduction, is that in online
440 games it is not possible to control the environment where participants undertake the
441 games (e.g. they may be distracted by other things happening around them), but this
442 should also simply add noise.

443

444 **QUANTIFICATION AND STATISTICAL ANALYSIS**

445 ***Colour divergence and background matching***

446 Collectively, we measured reflectance spectra of 155 leaves and 240 rocks from eight
447 populations. Each sample was measured three times to obtain an average value. To
448 examine phenotype divergence, we used MANOVA (multivariate analysis of variance)
449 to analyse the colour divergence among populations, with coordinates of colour loci
450 (L^* , a^* and b^*) as the dependent variables (with Pillai's trace to generate F -test). As
451 colour and luminance are often used separately in visual tasks, we also analysed the
452 chromatic (using a^* and b^* as dependent variables, MANOVA) and luminance (L^* ,
453 one-way ANOVA) dimensions, separately.

454 We used the colour distance between the focal plant and its background to
455 estimate the degree of background matching. Chromatic colour similarity and
456 luminance were analysed separately. As the real background is complex in the field,
457 and light conditions fluctuate, the chromatic dimension should be more important
458 for target detection. For chromatic similarity, distances between the loci (with a and

459 b coordinates in CIEL^{*}a^{*}b^{*} space) of each of the N plant and M rocks were calculated,
460 generating $N \times M$ distance values (see specific sample sizes of each population in
461 Table S1). These values were then averaged by plant individuals, creating the N
462 distances for each population. For luminance (lightness), the difference in L^* value
463 between colour loci was analysed using the same method (in the one-dimensional
464 condition). One-way ANOVAs were used to analyse the difference of these distances
465 among populations. All colour distance values were square-root-transformed to
466 improve homogeneity in analyses, the original values were shown in Figures S2.

467

468 ***Camouflage and harvest pressure correlation***

469 We used a Spearman ρ method to test the correlation between background
470 matching and the intensity of harvest pressure (in terms of collection intensity and
471 collection difficulty).

472

473 ***Factors influencing focal plant detection using an online experiment***

474 It is possible that the subjects failed to find the target because they were distracted
475 from the game temporarily. Therefore, trials that failed to find the target were not
476 included in the analysis (see also [11]). We noticed that sometimes players could
477 have been using a random scatter-gun strategy to locate the target successfully
478 instead of looking for the target visually. Such data were discarded by excluding data
479 for slides that received clicks more than three times. The final sample size of records
480 is 6,849 from 542 subjects (during Aug. 2018 to Nov. 2019).

481 We used a linear mixed model to examine whether the capture time was
482 influenced by the following fixed factors: target conspicuousness (saliency value),
483 visual condition (trichromatic or dichromatic images), player experience (whether
484 played the game before), screen size, distance of target from edge, distraction
485 (whether there is a distraction, the flowers of non-focal species), and trial number
486 (the number of slide shown to the player), with ID as a random effect. Capture time
487 and EdgeDistance were log-transformed to improve normality. The full model
488 contains all terms and their 2-way interactions, specified using function lmer

489 (package lme4 [31]) in R version 3.4.3 [32]. The model was then simplified by AIC
490 (Akaike information criterion) in a stepwise algorithm with the backward direction,
491 using **step** function. The simplified model contained all seven fixed terms and seven
492 2-way interactions.

493

494

495 **Supplementary Information:** Supplementary information includes one table, two
496 figures and a data file, and can be found with this article online at [XXX](#).

497

498 **Data S1. Original data. Related to Figure 2.**

499 This file includes eight data sheets. The sheet 'Spectra' includes reflectance spectra
500 of leaf (LF) and rock (RK) samples from eight populations. Sheet 'ColourModelRef'
501 contains parameters used in colour analyses. Sheet 'CoordinatesInCIELAB' contains
502 coordinates of colour loci in CIEL*a*b* space, calculated from spectral data. Sheet
503 'Distance-ab' contains mean chromatic colour distance (between leaves and rocks) in
504 CIEL*a*b* space in different populations. Sheet 'Differ-L' contains mean luminance
505 difference (between leaves and rocks) in CIEL*a*b* space in different populations.
506 Sheet 'Price' contains market price of *Fritillaria delavayi* bulbs from 2016 to 2020.
507 Sheet 'Abundance' contains plant abundance estimated by ten plots in each
508 population. Sheet 'Difficulty' contains time spend for digging out a single bulb in
509 each population.

510

511

512 **References**

- 513 1. Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E.,
514 Hill, G.E., Jablonski, N.G., Jiggins, C.D., Kelber, A., et al. (2017). The biology of
515 color. *Science* 357, eaan0221.
- 516 2. Kettlewell, H.B.D. (1955). Selection experiments on industrial melanism in the
517 Lepidoptera. *Heredity* 9, 323-342.
- 518 3. Darimont, C.T., Carlson, S.M., Kinnison, M.T., Paquet, P.C., Reimchen, T.E., and
519 Wilmers, C.C. (2009). Human predators outpace other agents of trait change
520 in the wild. *Proc. Natl. Acad. Sci. USA* 106, 952-954.

- 521 4. Sharpe, D.M., and Hendry, A.P. (2009). Life history change in commercially
522 exploited fish stocks: an analysis of trends across studies. *Evol. Appl.* 2, 260-
523 275.
- 524 5. Law, W., and Salick, J. (2005). Human-induced dwarfing of Himalayan snow
525 lotus, *Saussurea laniceps* (Asteraceae). *Proc. Natl. Acad. Sci. USA* 102, 10218-
526 10220.
- 527 6. Lev-Yadun, S. (2016). *Defensive (anti-herbivory) coloration in land plants*,
528 (Switzerland: Springer).
- 529 7. Niu, Y., Sun, H., and Stevens, M. (2018). *Plant Camouflage: Ecology, Evolution,*
530 *and Implications. Trends Ecol. Evol.* 33, 608-618.
- 531 8. Strauss, S.Y., and Cacho, N.I. (2013). Nowhere to run, nowhere to hide: the
532 importance of enemies and apparency in adaptation to harsh soil
533 environments. *Am. Nat.* 182, E1-E14.
- 534 9. Niu, Y., Chen, Z., and Stevens, M. (2017). Divergence in cryptic leaf colour
535 provides local camouflage in an alpine plant. *Proc. Royal. Soc. B* 284,
536 20171654.
- 537 10. Farmer, E.W., and Taylor, R.M. (1980). Visual search through color displays:
538 Effects of target-background similarity and background uniformity. *Percept.*
539 *Psychophys.* 27, 267-272.
- 540 11. Troscianko, J., Wilson-Aggarwal, J., Griffiths, D., Spottiswoode, C.N., and
541 Stevens, M. (2017). Relative advantages of dichromatic and trichromatic color
542 vision in camouflage breaking. *Behav. Ecol.* 28, 556-564.
- 543 12. Curtis, P.D., Curtis, G.B., and Miller, W.B. (2009). Relative resistance of
544 ornamental flowering bulbs to feeding damage by voles. *Horttechnology* 19,
545 499-503.
- 546 13. Festa-Bianchet, M., and Mysterud, A. (2018). Hunting and evolution: theory,
547 evidence, and unknowns. *J. Mammal* 99, 1281-1292.
- 548 14. Dimitrova, M., and Merilaita, S. (2011). Prey pattern regularity and
549 background complexity affect detectability of background-matching prey.
550 *Behav. Ecol.* 23, 384-390.
- 551 15. Xiao, F., and Cuthill, I.C. (2016). Background complexity and the detectability
552 of camouflaged targets by birds and humans. *Proceedings of The Royal*
553 *Society B: Biological Sciences* 283, 20161527.
- 554 16. Bukovac, Z., Shrestha, M., Garcia, J.E., Burd, M., Dorin, A., and Dyer, A.G.
555 (2017). Why background colour matters to bees and flowers. *J. Comp. Physiol.*
556 *A* 203, 369-380.
- 557 17. McElroy, J.S. (2014). Vavilovian mimicry: Nikolai Vavilov and his little-known
558 impact on weed science. *Weed Sci.* 62, 207-216.
- 559 18. Xu, B., Sun, H., and Li, Z.-M. (2014). *Seed plants of the alpine subnival belt*
560 *from the Hengduan mountains, SW China*, (Beijing: Science Press).
- 561 19. Chinese Pharmacopoeia Commission (2010). *Pharmacopoeia of the People's*
562 *Republic of China*, (Beijing: China Medical Science Press).
- 563 20. Cunningham, A.B., Brinckmann, J.A., Pei, S.J., Luo, P., Schippmann, U., Long,
564 X., and Bi, Y.F. (2018). High altitude species, high profits: Can the trade in wild

- 565 harvested *Fritillaria cirrhosa* (Liliaceae) be sustained? *J. Ethnopharmacol.* **223**,
566 142-151.
- 567 21. Xin, G.-Z., Lam, Y.-C., Maiwulanjiang, M., Chan, G.K., Zhu, K.Y., Tang, W.-L.,
568 Dong, T.T.-X., Shi, Z.-Q., Li, P., and Tsim, K.W. (2014). Authentication of *Bulbus*
569 *Fritillariae Cirrhosae* by RAPD-derived DNA markers. *Molecules* **19**, 3450-
570 3459.
- 571 22. Stockman, A., and Sharpe, L.T. (2000). The spectral sensitivities of the middle-
572 and long-wavelength-sensitive cones derived from measurements in
573 observers of known genotype. *Vision Res.* **40**, 1711-1737.
- 574 23. International Commission on Illumination (2004). *Colorimetry* (Third Edition).
- 575 24. Sharma, G., and Bala, R. (2013). *Digital Color Imaging Handbook* (2 ed.), (CRC
576 Press).
- 577 25. Sharma, G., Wu, W., and Dalal, E.N. (2005). The CIEDE2000 color-difference
578 formula: Implementation notes, supplementary test data, and mathematical
579 observations. *Color Res. Appl.* **30**, 21-30.
- 580 26. Nokelainen, O., Maynes, R., Mynott, S., Price, N., and Stevens, M. (2019).
581 Improved camouflage through ontogenetic colour change confers reduced
582 detection risk in shore crabs. *Funct Ecol* **33**, 654-669.
- 583 27. Galloway, J.A., Green, S.D., Stevens, M., and Kelley, L.A. (2020). Finding a
584 signal hidden among noise: how can predators overcome camouflage
585 strategies? *Philos. Trans. R. Soc. Lond. B* **375**, 20190478.
- 586 28. Pike, T.W. (2018). Quantifying camouflage and conspicuousness using visual
587 salience. *Methods Ecol. Evol.* **9**, 1883-1895.
- 588 29. Itti, L., Koch, C., and Niebur, E. (1998). A model of saliency-based visual
589 attention for rapid scene analysis. *IEEE Trans. Pattern Anal. Machine Intell.* **20**,
590 1254-1259.
- 591 30. Sherratt, T.N., Pollitt, D., and Wilkinson, D.M. (2007). The evolution of crypsis
592 in replicating populations of web-based prey. *Oikos* **116**, 449-460.
- 593 31. Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann,
594 H., Dai, B., Grothendieck, G., Green, P., and Bolker, M.B. (2015). Fitting linear
595 mixed-effects models using lme4. *J. Statistical Software* **67**, 48.
- 596 32. R Core Team (2017). *R: A language and environment for statistical computing*.
597 *Stat. Comput.* Vienna, Austria. URL <http://www.R-project.org>.