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RESEARCH ARTICLE

REVISED Wild snapdragon plant pedigree sheds light on limited connectivity enhanced by higher migrant reproductive success in a fragmented landscape

[version 2; peer review: 2 approved]

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Abstract

Background: In contrast with historical knowledge, a recent view posits that a non-negligible proportion of populations thrive in a fragmented landscape. One underlying mechanism is the maintenance of functional connectivity, i.e., the net flow of individuals or their genes moving among suitable habitat patches. Alternatively, functional connectivity might be typically limited but enhanced by a higher reproductive success of migrants.

Methods: We tested for this hypothesis in wild snapdragon plants inhabiting six patches separated by seawater in a fragmented Mediterranean scrubland landscape. We reconstructed their pedigree by using a parentage assignment method based on microsatellite genetic markers. We then estimated functional connectivity and the reproductive success of plants resulting from between-patch dispersal events.

Results: We found that wild snapdragon plants thrived in this fragmented landscape, although functional connectivity between habitat patches was low (i.e. 2.9%). The progeny resulting from between-patch dispersal events had a higher reproductive success than residents.

Conclusion: Our findings imply that low functional connectivity in a fragmented landscapes may have been enhanced by higher reproductive success after migration. This original mechanisms might be partly compensating the negative impact of fragmentation.

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Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Functional connectivity, dispersal, wild population pedigree, fragmented landscape

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REVISED **Amendments from Version 1**

This revision was the opportunity to clarify that we do not think snapdragon plant populations are benefiting directly from habitat fragmentation. Our aim was, and still is, to point at a remarkable mechanism whereby in the presence of limited connectivity the high reproductive success of migrants makes this limited connectivity more likely to be effective.
As suggested by the reviewers, we also discuss the potential scenarii underlying the current status of genetic differentiation between patches, which may result from an evolutionary equilibrium or might be currently in the process of being resolved. As mentioned in the text, our interest for identifying connectivity mechanisms in these plants comes from their ability to perform well in the fragmented landscape, as illustrated by their demographic expansion. As suggested by the reviewers, we added more information in a new table in the results about the percentage of ghost (unsampled) parents per patch. We also clarified the legend of Table 1, changed the resolution of Figure 4 and added in the materials and methods how we obtained the p-values for Hs. In addition, we nuanced our discussion about the extent to which connectivity was weak. In fact, our aim always was to point out that this amount of connectivity, which is low in comparison to examples from the literature, was rendered more effective by the higher reproductive success of between-patch mating events. Finally, following the reviewers' suggestion, we discuss a little more the mechanisms underlying this high reproductive success of migrants but took care not to enter into much details as to avoid speculation.

Any further responses from the reviewers can be found at the end of the article

Introduction

Human-induced or natural habitat fragmentation has long been known to adversely affect population persistence and distribution (Fahrig & Merriam, 1994). In a fragmented habitat, theory predicts that the limited movement of individuals or limited gene exchanges among habitats enhances reproductive isolation and opens the way to genetic drift and loss of genetic diversity in small populations (Aguilar *et al.*, 2008; Gittleman *et al.*, 2000; Vranckx *et al.*, 2012). As a consequence, the long-term survival and adaptive potential of populations is expected to decline in a fragmented habitat (Frankham, 1995; Wade *et al.*, 2017). However, a recent meta-analysis demonstrated that a non-negligible part of populations thrived in fragmented habitats (Fahrig, 2017). For example, a higher abundance of sawtooth grain beetle and a higher species richness in ant communities were found in fragmented rather than continuous habitats (Bancroft & Turchin, 2003; Dauber *et al.*, 2006). The mechanisms underlying the resilience of populations in fragmented landscapes remain poorly understood. (Fahrig, 2017; Fahrig *et al.*, 2019).

Maintenance of high functional connectivity (i.e., the net flow of organisms and their genes moving among suitable habitat patches, Tischendorf & Fahrig, 2000) counteracting reproductive isolation might explain why some populations thrive in a fragmented habitat (Dick *et al.*, 2003; Nason & Hamrick, 1997). To date, functional connectivity has been widely evaluated by using estimates of dispersal rates and genetic differentiation (Auffret *et al.*, 2017). The flow of individuals or genes can only counteract reproductive isolation in the long term if migrant individuals successfully reproduce and migrant genes are successfully transmitted to the next generations (a mechanism known as effective connectivity; Cayuela *et al.*, 2018; Robertson *et al.*, 2018). For example, in snail kite populations characterised by a high dispersal rate, and therefore high functional connectivity, effective connectivity was limited because migrants had a low reproductive success (Robertson *et al.*, 2018). Conversely, populations characterised by limited dispersal rates but thriving in a fragmented habitat might have maintained effective connectivity through high migrant reproductive success (Lowe & Allendorf, 2010). This latter hypothesis remains poorly tested, as few studies jointly assessed connectivity and migrant reproductive success in the wild (but see Robertson *et al.*, 2018; Robertson *et al.*, 2019; Vasudev & Fletcher, 2016).

In plants, functional connectivity involves the dispersal of seed and pollen, resulting in the establishment of a new adult plant (i.e., effective dispersal, Auffret *et al.*, 2017; Schupp *et al.*, 2010). Despite the use of parentage assignment in plant functional connectivity studies (Kamm *et al.*, 2010; Moran & Clark, 2011; Sork *et al.*, 1999), which offers the opportunity to assess the reproductive success of these new plants, their reproductive success has received little attention (Aguilar *et al.*, 2019; Auffret *et al.*, 2017). One reason for this is the need for a pedigree based on a long-term study. Here, we reconstructed a ten-year multigenerational pedigree of wild snapdragon plants (*Antirrhinum majus* L.) thriving in a fragmented landscape in southern France. These snapdragon plants inhabit patches of suitable Mediterranean scrubland isolated for a few hundred meters by seawater, which corresponds to the remains of a site where salt was manufactured by exploiting semi-natural crystallisation ponds. Here we assessed jointly functional connectivity between patches and the reproductive success of plants.

Seed dispersal by small animals and insects can generally reach ca. hundred meters (Uroy *et al.*, 2019) and up to several kilometres when large or migratory animals are involved (Mueller *et al.*, 2014; Vittoz & Engler, 2007). In snapdragon plants, seed dispersal occurs by gravity and should therefore be geographically-limited. We therefore did not expect seed dispersal to connect patches of land separated by seawater. Pollen dispersal might enable connectivity because snapdragon plants are pollinated by bumblebees, carpenter bees and other large-sized pollinators known to fly distances larger than the distance separating patches in this fragmented landscape (Chapman *et al.*, 2003). However, the distance covered by these pollinators is potentially limited in a fragmented landscape, even at an extremely small spatial scale, e.g., across distances of ca. 40m (Goverde *et al.*, 2002). Although landscape fragmentation may affect forests at the scale of kilometres, it might affect insect pollinated plants at the scale of a few hundred meters. We therefore expect to find typically limited functional connectivity and possibly genetic differentiation between these patches of snapdragon plants thriving in a fragmented landscape. We tested for the rarely explored hypothesis that connectivity would be effective on the long term; in other words, that the progeny resulting from migration between patches would successfully reproduce.

Results

Demographic expansion of snapdragon plants in a fragmented landscape

Over ten years (2010–2019), we sampled ca. twelve thousand flowering plants (N=12594) on six patches (from 2508 to 12547m 2 area) isolated from each other by a few hundred meters of seawater (from 158 to 1627m, Table 1). Our long-term survey data showed that these snapdragons are ongoing a demographic expansion. The population size has increased ten-fold in ten years, with an average annual population growth rate (λ) ranging from 1.25 for the easternmost patch (Patch 6) to 1.75 for the patch3 and 5 (Table 2).

High genetic diversity and low genetic differentiation

Genetic diversity (estimated by Nei's expected heterozygosity and associated standard error; Hs±SE) was high in every patch of snapdragon plants in this fragmented landscape ranging from 0.678 for patch 4 to 0.707 for patch 5 (average Hs=0.69 ± 0.004). These diversity values are similar to those previously found in populations distributed across the species geographic range (average Hs=0.65±0,02; Pujol *et al.*, 2017). Other genetic parameters (e.g., allelic richness) corroborating this high diversity can be found in Table 3). We also found low but significantly different from zero genetic differentiation amongst patches (Fst=0.04, p=0.001). Fst between pairs

Table 1. Mean distance between and within patches in meters. On the diagonal is the mean distance between individuals within a patch, in meters. Off-diagonal is the mean distance between individuals of the different patches, in meters.

	Patch 1	Patch 2	Patch 3	Patch 4	Patch 5	Patch 6
Patch 1	94.8					
Patch 2	579.9	47.6				
Patch 3	947.9	407.5	23.5			
Patch 4	1177.7	630.8	229.9	40.5		
Patch 5	1570	1014.9	622.1	392.6	46.2	
Patch 6	1627.9	1057	690.1	472	158.7	20.4

Table 2. Growth rates per patch and year. Population growth rates >1 (reflecting expansion) are in bold. If there was no individual sampled or only one individual sampled in a patch on a given year, the growth rate of the subsequent year could not be calculated. In this case, we reported the number of plants, in italics between brackets. Caution should be taken when interpreting patch 1 growth rates (see methods). Patch 1 growth rates are indicated for information only.

	patch					
growth rate	**1**	**2**	**3**	**4**	**5**	**6**
Median	NA	**1.32**	**1.75**	**1.41**	**1.75**	**1.25**
2011	*[11]*	*[43]*	*[1]*	*[28]*	**3.17**	**1.4**
2012	*[0]*	**1.4**	**8**	**1.36**	0.08	0.57
2013	*[161]*	**6.72**	**7.5**	**2.53**	**14.5**	**5.5**
2014	0.04	0.22	0.13	0.58	**1.11**	0.22
2015	**78**	**4.69**	**14.88**	**1.46**	**3.46**	**5.05**
2016	0.92	**2.12**	**2.16**	**4.26**	**1.86**	**2.33**
2017	**2.04**	0.6	0.32	0.21	0.7	0.49
2018	0.18	0.64	0.98	0.73	**1.75**	**1.12**
2019	**3.02**	**1.23**	**1.33**	**2.04**	0.91	1.25

of patches ranged from 0.014 to 0.079 ($p<0.001$ for all pairwise Fst estimates; Table 4). We did not observe any identifiable trend in time or space shaping Fst (Figure 1).

Table 3. Descriptive statistics for microsatellites markers. It includes the name of the locus (Locus), allelic richness (A), number of plants genotyped at a given marker (N), observed heterozygosity (Hobs), expected heterozygosity (Hexp), polymorphic information content (PIC), Parent-pair exclusion probability (PPexp). Estimates were calculated on data pooled among years and patches.

Locus	A	N	Hobs	Hexp	PIC	PPexp
Antibg36	17	12486	0.848	0.880	0.868	0.910
Antibg38	6	12541	0.594	0.608	0.533	0.488
Antibg23	7	12539	0.546	0.579	0.545	0.558
Antibg40	3	12546	0.642	0.658	0.584	0.513
Antibg11	19	12526	0.831	0.829	0.811	0.853
Antibg18	6	12542	0.587	0.604	0.559	0.552
Antibg03	17	12512	0.633	0.659	0.627	0.656
Antibg02	10	12516	0.773	0.809	0.785	0.815
Antibg10	29	12317	0.642	0.842	0.824	0.864
Antibg12	10	12550	0.653	0.65	0.597	0.564
Antibg20	9	12494	0.691	0.723	0.80	0.687
Antibg30	29	12470	0.875	0.925	0.920	0.961
Antibg29	7	12545	0.564	0.576	0.494	0.436
Antibg33	24	12511	0.827	0.888	0.878	0.926
Antibg39	9	12536	0.576	0.598	0.569	0.591
Antibg22	6	12517	0.691	0.742	0.699	0.692
Antibg14	17	12503	0.855	0.893	0.884	0.928
Antibg27	6	12557	0.457	0.461	0.385	0.326

Table 4. Pairwise Fst values between patches estimated in 2019. All p-values are <0.0001.

Patch	1	2	3	4	5
2	0.042	-			
3	0.079	0.049	-		
4	0.067	0.043	0.015	-	
5	0.048	0.035	0.035	0.022	-
6	0.064	0.048	0.034	0.029	0.014

Conservative parentage assignment and missing parentage links

The long-term survey of snapdragon plants allowed us to build a multigenerational pedigree including ca. 40% of the genotyped plants (N=5053; Figure 2). For 23% of the genotyped plants, both parents were assigned (n=2818 parent-offspring triads). We used greatly effective markers to reconstruct the pedigree (cumulated markers probability to exclude a 'random' individual from parentage, Wang, 2007, PPexp>0.999, see Table 3 for more details). Our assignment approach was highly selective. We only included parents with high assignment probability (above 95%) in the pedigree. Although this final pedigree is highly conservative, it comprises a large number of plants and family links; 5053 individuals with 2235 founders, 2818 offspring with two identified parents (for which 2815 have spatial coordinates), 805 offspring with only one identified parent, 2571 parents, 420 full-sibling links, 17170 half-sibling links (see Table 5 and Figure 2), which we except to be a representative sample at the scale of the six patches. These plants are grouped in 234 families spanning across one to five generations (Figure 2B) and composed on average by 21.5 individuals. The non-negligible part of plants with only one or no assigned parents was likely mothered or fathered by plants that we did not sample (Table 6). Plants measured at the beginning of the survey probably had parents from before the sampling campaign (e.g., 94 % of unassigned parents in 2012 against 52% in 2019). In addition, some parents were likely missed in the surveyed area during fieldwork even if we conducted a thorough search of sexually mature plants in the area. ww might also likely be located outside the surveyed area. The presence of plants with at least one unidentified parent suggests migration from outside the studied area (Bacles *et al.*, 2006; Sebbenn *et al.*, 2011).

Low functional connectivity between the six patches

Functional connectivity was estimated by the rate of between-patch effective dispersal events (Auffret *et al.*, 2017). The use of the multigenerational pedigree in combination with the spatial coordinates of the plants revealed that only 2.9% of offspring had one parent on a different patch (n=81 out of a total of 2818 offspring with two known parents, details per patch in Figure 3 and Table 7). This small proportion likely indicates low functional connectivity through effective pollen dispersal events among patches. On average, the pollen dispersal distance between patches was 345m (ranging from 99 to 1656m). In addition, we assumed that dispersal events between patches allowed us to identify the maternal parent located on the same site as the offspring. We estimated an average seed dispersal distance of 3.86m (ranging from 0.22 to 31.28m, Figure 4). Dispersal distance within patches ranged from 0.02 to 256.17m, including seed and pollen. Dispersal distances between patches were not necessarily higher than within patches (e.g., larger distances are found within patch 5 than between patch 5 and 6 or 3 and 4, Table 1). Yet only 37 dispersal events within patches out of 5359 (0.7%) occurred at a similar spatial scale than between-patch dispersal events (Figure 4).

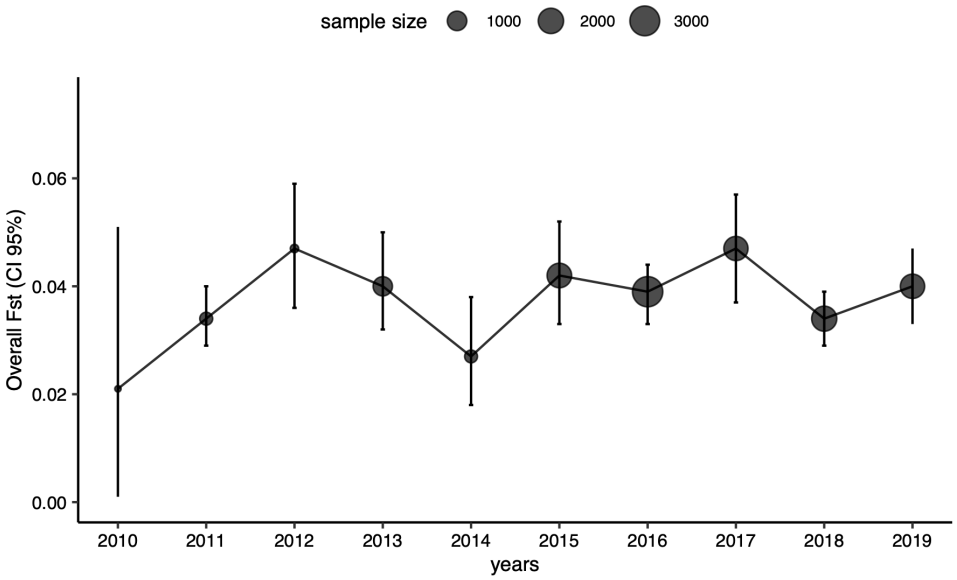


Figure 1. Genetic differentiation (multilocus Fst) amongst patches over 10 years. Dots and bars around the dots correspond respectively to global Fst estimates measured amongst patches and their associated 95% confidence intervals. Dot size is proportional to the sample size used to estimate Fst.

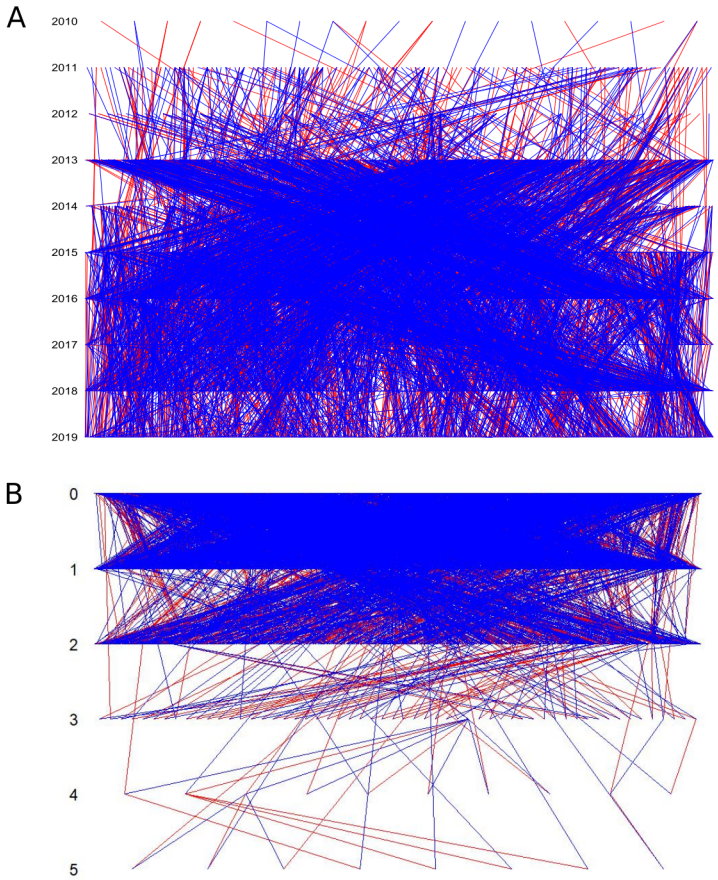


Figure 2. Pedigree represented per year (**A**) and by generations (**B**). Each line represents a parent-offspring link. Red and blue lines represent the closest and the farthest parents, respectively.

Table 5. Pedigree description. The final pedigree is only composed of parent-offspring triads.

	Quantity
Number of individuals in the pedigree	5053
Parent-offspring links	5636
Full sibling links	420
Half-sibling links	17170
Grand-parent offspring links	1362
Full avuncular links	86
Full first cousin links	56
Half avuncular links	4596
Number of founders	2235
Maximum pedigree depth (in generations)	5

Table 6. Proportion of plants with one or two-unidentified parents per patches and year. Due to *A.majus* biology and the beginning of the study, we identified parents only from 2012.

Patches	1	2	3	4	5	6
2 unidentified parents						
2012	NA	0.96	1	0.86	0.92	1
2013	0.92	0.88	0.91	0.77	0.83	0.87
2014	0.88	0.89	1	0.8	0.76	0.56
2015	0.81	0.75	0.65	0.65	0.7	0.75
2016	0.72	0.62	0.69	0.72	0.74	0.66
2017	0.75	0.64	0.45	0.68	0.69	0.63
2018	0.76	0.58	0.51	0.43	0.68	0.54
2019	0.61	0.52	0.38	0.47	0.6	0.54
Median	0.77	0.67	0.65	0.67	0.71	0.64
Patches	**1**	**2**	**3**	**4**	**5**	**6**
1 unidentified parent						
2012	NA	0.04	0	0.1	0.08	0
2013	0.08	0.07	0.07	0.16	0.12	0.09
2014	0.13	0.08	0	0.09	0.12	0
2015	0.11	0.07	0.15	0.15	0.11	0.11
2016	0.14	0.07	0.04	0.1	0.03	0.09
2017	0.11	0.07	0.18	0.21	0.06	0.04
2018	0.03	0.03	0.04	0.03	0.03	0.03
2019	0.06	0.03	0.01	0.08	0.03	0
Median	0.11	0.07	0.04	0.10	0.06	0.04

Little spatial and temporal variation in connectivity

Functional connectivity varied both spatially and temporally, but to a low extent. It increased from 2.8% in 2014 to 4.5% in 2016 and finally decreased to 1.4% in 2019.

At the spatial level, it decreased with the between-patch geographical distance (rho=-0.75, p=0.001), ranging from 0% between several pairs of patches separated by distance ranging from 472m to 1627.9m; to 1.9% between patches 5 and 6 separated by 158.7m (Figure 3).

Effective connectivity

Functional connectivity between patches was effective. We found that 64% of plants resulting from between-patch dispersal events successfully reproduced over the duration of the survey. Plants resulting from between-patch dispersal events had on average 0.79 offspring per plant. These plants had around twice as many offspring as plants with two resident parents that had on average 0.43 offspring (regression model parameter back-transformed estimate: 2.151, CI95%: 1.05-4.41, p=0.036).

Discussion

Our survey revealed that thousands of snapdragon plants were thriving on patches of Mediterranean scrubland interrupted by seawater ponds formerly exploited for salt production. We found that these plants were undergoing demographic growth and characterized by high levels of genetic diversity that were similar to those of continuous populations at similar spatial scales (Pujol *et al.*, 2017; Ringbauer *et al.*, 2018). The good condition of these snapdragon plants makes it an interesting system for investigating the mechanisms by which populations are not adversely affected in a fragmented landscape. The low but statistically significant genetic differentiation between patches, which illustrates the balance of evolutionary forces that are migration and genetic drift, provided an ambiguous message about the potential actual reproductive isolation of patches. This result could be explained by genetic drift being at equilibrium with migration, with genetic drift caused by a low effective population size resulting from a small number of founders and migration being limited as a result of weak connectivity. Genetic differentiation would then reflect a history of patches drifting apart. An alternative scenario is that of evolutionary change, where this same divergence reflected by genetic differentiation would be in the process of being resolved by recent migration resulting from effective connectivity. We used a connectivity approach based on the reconstruction of a multigenerational pedigree to clarify whether these snapdragon plants were reproductively isolated in this fragmented landscape.

Low functional connectivity across the fragmented landscape

Our results showed that 2.9% of offspring resulted from dispersal events between patches of snapdragon plants, which is coherent with our genetic differentiation results (e.g. one migrant per generation rule, Whitlock & McCauley, 1999; Mills & Allendorf, 1996). Similar low percentages have previously been found in other species inhabiting fragmented landscapes and were considered to indicate limited functional

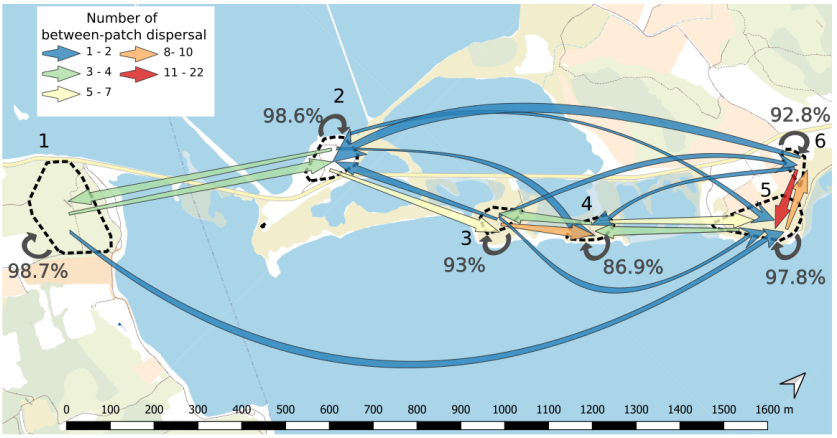


Figure 3. Connectivity map between patches. Each patch is delimited by dashed line and its corresponding number going from 1 to 6. Arrows indicate the presence of effective pollen dispersal from a given patch to another. The number of between-patch dispersal events ranges from 1 to 22 events and the colour of arrows represents this number (from blue to red, see legend on the figure). Circular arrows on each patch represent self-recruitment and are accompanied by the corresponding percentage. Background map is provided freely by IGN at https://www.geoportail.gouv.fr/donnees/plan-ign-v2.

Table 7. Functional connectivity per patch all years combined. Outbound connectivity is the percentage of offspring on a different patch with the farthest parent on a given focal patch. Inbound connectivity is the percentage of offspring on the given focal patch with the farthest parent on a different patch. Total connectivity is the sum of inbound and outbound connectivity.

Patch	1	2	3	4	5	6
Outbound connectivity	1.7%	2.3%	8.6%	8.1%	1%	16%
Inbound connectivity	1.3%	1.4%	6.9%	13%	2%	7.2%
Total functional connectivity	2.95%	3.69%	14.45%	19.29%	2.98%	20.86%

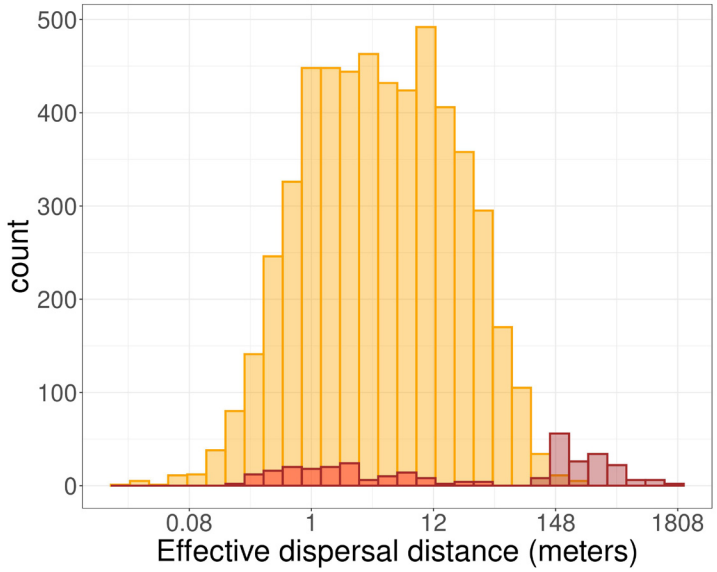


Figure 4. Distributions of the effective dispersal distances. Yellow bars represent dispersal distance between parent-offspring triads inhabiting the same patch. Bars with brown borders represents distance between parent-offspring triads, with one parent inhabiting on a different patch. Brown-bordered bars filled in red represent the distance between the closest parents and the offspring, which is more likely representing seed dispersal, while brown-bordered bars filled in brown represent the distance between the farthest parents and the offspring.

connectivity (Mueller *et al.*, 2014; Sebbenn *et al.*, 2011). Higher connectivity – up to an order of magnitude greater – has also been found in other species inhabiting fragmented landscapes (Bacles & Ennos, 2008; Hebel *et al.*, 2007; Wang *et al.*, 2010). It is therefore legitimate to consider that our results show functional connectivity between snapdragon plant patches. However, our results also outlined that connectivity is limited in this fragmented Mediterranean scrubland landscape where ecological barriers such as the seawater ponds separating the patches might have limited the movement of pollinators. Bumblebees and carpenter bees are the main pollinators of snapdragon plants (Tastard *et al.*, 2012). They are known to cover long distances (Chapman *et al.*, 2003). For instance, we detected pollen dispersal events between patches over distances up to 1656 meters. However, pollinators able to cover long distances rarely do so frequently (Walther-Hellwig & Frankl, 2000; Zurbuchen *et al.*, 2010). Pollination might have typically been favoured between neighbouring plants 2 (Kunin, 1997; Tastard *et al.*, 2012). Our findings therefore did not support one of the possible explanations put forward for populations thriving in fragmented landscapes, namely the presence of high functional connectivity (Fahrig, 2017; Fahrig *et al.*, 2019).

Higher reproductive success associated with connectivity

Reconstructing the multigenerational pedigree of wild populations is challenging but rewarding because it also provides information about the reproductive success of plants. Our results showed that plants resulting from between-patch dispersal events had higher reproductive success than plants with resident parents. While this might be the result of random processes (e.g. colinisation waves, Excoffier *et al.*, 2009), organisms colonising new habitats (e.g., geographic range expansion, biological invasion) are expected to have a higher fitness than the average fitness of resident organisms (Bonte *et al.*, 2014; Ronce, 2007). Organisms inhabiting fragmented landscapes can also be selected for their potential to invade other patches (e.g, plant height, seed mass Williams *et al.*, 2019). Our findings highlight the knowledge gained by evaluating offspring quality beyond the count of their number (Aguilar *et al.*, 2019). Although functional connectivity was low, migrant genes from other patches were therefore successfully integrated into the resident gene pool. Our findings contrast with a study in endangered birds where functional connectivity remained high in the fragmented landscape but the reproductive success was low, which was detrimental to the species (Robertson *et al.*, 2018; Robertson *et al.*, 2019). Our approach in the wild cannot identify the mechanisms underlying the higher reproductive success of snapdragon plants resulting from between-patch dispersal events nor the causal relationship between this reproductive success and population demographic expansion. However, it exposed a remarkable aspect whereby low functional connectivity in fragmented landscapes is at least partly effective because of a high migrant reproductive success. The high reproductive success of migrants likely participates to compensate the negative impact of reproductive isolation. The links between this mechanism and population growth remains to be explored.

Conclusion

Our study in snapdragon plants adds up to the recent awareness that some species have the potential to thrive in a fragmented landscape. Our findings support an often-neglected hypothesis whereby typical low connectivity in a fragmented landscape might be rendered more effective by a higher reproductive success of migrants. The extent to which migrants successfully transmit their genes to the next generations is rarely evaluated (Aguilar *et al.*, 2019; Robertson *et al.*, 2018). Our findings highlight the benefit of integrating the reproductive success of migrants in studies evaluating connectivity in a fragmented landscape.

Methods

Study population and data collection

Snapdragon plants (*Antirrhinum majus* L., Plantaginaceae) are short-lived, herbaceous perennials. Their geographic distribution is restricted to southern Europe, over the eastern half of Pyrenees Mountains, and extending south and north along the Mediterranean coast from Barcelona to Montpellier (Khimoun *et al.*, 2011). They grow in a variety of environments, including Mediterranean scrubland, scree, understorey vegetation, grassland meadows and sparse shrubland (Khimoun *et al.*, 2013). They are hermaphroditic, self-incompatible, and produce annual inflorescences with zygomorphic flowers pollinated mainly by bumblebees (*Bombus spp*) and carpenter bees (*Xylocopa violacea*) and small seeds dispersed by gravity a few meters apart from the plant (Andalo *et al.*, 2010)

Here we focus on snapdragon plants located in a Mediterranean scrubland ecosystem in southern France; between Bages (Latitude: 43.1167; Longitude: 2.9833) and Peyriac de Mer (Latitude: 43.0833; Longitude: 2.9667). Those plants persist on six small isolated rocky hills separated by salt lakes that used to be “Saline d’Estarac”; a site where salt was manufactured from crystalisation ponds using solar evaporation between the years 1007 and 1940 (Dupont, 1958; Larguier, 2014). As a consequence, this fragmented landscape constrains plants to a patchy distribution.

Between 2010 and 2019, we monitored wild snapdragon plants in the six patches isolated by a few hundred meters of seawater. All patches (numbered 1 to 6 from southwest to northeast) were surveyed between June and early July when plants are sexually mature and the reproductive season ends. All sexually mature snapdragon plants were identified after a thorough search of the area of the six patches (n=12594). Four leaves per plant were sampled for DNA extractions. The geographic location of most plants (12495 out of 12594) was recorded using a GNSS receiver (GNSS device Geo7X, Trimble, Westminster, USA) that provided us with high precision coordinates (sub-meter precision) after the data were post-processed by comparison with data from an independent monitoring station. Based on the barycenter of plant coordinates within a given patch, we calculated the mean distance between patches in meters. We also calculated the mean distances separating plants within patches. All plants, including those without geographical location (n=99), have a patch number corresponding to their location.

To assess the demography of snapdragon plants, we estimated the annual growth rate in every patch as $\lambda = N_{T-1}/ N_T$, where N_T is the number of individuals in the current year and N_{T-1} the number of individuals in the previous year. If $\lambda>1$ the population is increasing, if $\lambda<1$ the population is decreasing, if $\lambda=1$ the population is stable (Pradel, 1996). Caution should be taken with patch 1 growth rates. We reported these values for information only. They are not interpretable per se because as the sampling strategy area might have varied form one year to the next (notably in 2015) due to the spatial heterogeneity of the patch. We do not expect this issue with patch 1 growth rates to interfere with genetic analyses.

DNA extraction and genotyping

DNA was extracted from leaf tissue using the Nucleospin 96 Plant II (Macherey Nagel, Hoerdt, France). After DNA extraction, samples were amplified at 20 polymorphic microsatellite loci (Debout *et al.*, 2012) using 3 multiplexes (A, B, C, Table 7). Each PCR was performed on a 10 µL total volume: 2 µL of a DNA extract; 3.5 µL QIAGEN Multiplex PCR Kit (Qiagen, Venlo, Limburg, The Netherlands); 0.4 to 0.6 µL of primer mix solution (Eurofins Genomics, Luxembourg, Luxembourg, depending on the multiplex see Table 7) and the remaining volume was completed with DNA-free water. PCRs were performed using a Mastercycler pro Thermal Cyclers (Eppendorf, Hamburg, Germany) with the following protocol for each multiplex: an initial denaturation step at 94°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds; 56°C (primer-specific annealing temperature) for 135 seconds; 72°C for 30 seconds; and a final extension at 60°C for 30 minutes. PCR products were sent to the Genoscreen DNA sequencing platform (Lille, France) where samples were analysed on an Automated Capillary DNA Sequencer (ABI 3730, Applied Biosystems, Foster City, CA, USA) using 2 µL of multiplexed PCR products, which were added to 7.75 µL of Hi-Di Formamide and 0.25 µL of the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GENEIOUS version 9.1 software (Biomatters, Auckland, New Zealand) and double cross-checked. We ensured that there were no genotype duplicates with the R package Allelematch (Galpern *et al.*, 2012). Finally, we ensured marker quality by keeping loci with less than 5% missing data (n=18 microsatellites), and kept individuals with less than 10% missing data (n=12428) for further analysis.

Population genetic differentiation

We estimated the genetic diversity of snapdragon plants in the six patches by using estimates of expected heterozygosity (Hexp), observed heterozygosity (Hobs), polymorphic information content (PIC), and allelic richness (A) for each markers. We also used Nei's multilocus estimates of expected heterozygosity with its standard error , within patch (Hs±SE). Analyses were conducted with the FRANz software (Riester *et al.*, 2009; Riester *et al.*, 2010).

We estimated genetic differentiation between pairs of patches by calculating Wright's fixation indices (Fst) using Weir and Cockerham's θST estimates (1984) in GENODIVE v2.0 (Meirmans & Van Tienderen, 2004). For genetic differentiation analyses, we removed markers that deviated significantly from Hardy-Weinberg equilibrium (all years confounded, n=7

Table 8. Primer sequences for 20 microsatellites loci in the *A.majus population*. For more details on the markers see Debout *et al.*, 2012.

Locus	*Primer sequences (5'-3')*	*Multiplex markers sets*
Antibg11	ATCAACCTGCATCACACCTG TGAATTACGTGAGCGTCGTC	A
Antibg23	TCATCACATTTCAATTCATCACA TTGCTTGCTCCAAGTGTTTG	A
Antibg32	GATCCGTGAGGAGTGTGGTT CGGCAATCTAATCTCCGAAA	A
Antibg36	TGCGTTAGATGATTGCCAAA AAGCTTCCGCTACGTCAGTT	A
Antibg38	CCAAGGAGAAGAAAATGTGAGG ATTAGGGAACCTCCAACGCT	A
Antibg40	CTCCTCTTCTCACCCGACAT CCCCTCCCTTTCCTAGTTCTT	A
Antibg02	TCTGGCAGCAAAAGGTAGAAA CGTGGGAGTTGAAGGAATGA	B
Antibg03	TTCTTCAAAGGCAAGCAGGT CATGCTCCTCGTGTGGAAT	B
Antibg10	AAACGCATATCCAAAGCAGG CGAAGACCTGCATGACAAAC	B
Antibg12	GCATGAAGCCCTGGAAATAA CTCAATGTGACAACTGCATCA	B
Antibg18	TTTGCTTTATGTCTTGGTCACCT GACGTGGTGATCAGCTAGGA	B
Antibg20	AACCAACAAAGCGAACAACC ATTCGTGACCGTAGAGACCG	B
Antibg21	AACTGGGTTTCCTTTCCCAG TTGAGAAATTACCATCATTGTTGTC	B
Antibg14	GAGGAAGCGATATCAAGGTATGA TGCTGCCTCCATACAGAAGA	C
Antibg22	TTCATCGAATTCTTCGTTCG AAACAACGCAATCCGATCTC	C
Antibg27	CGTCGCTAGTTTTCAGCCTC AAATGGTTGCATCCTCCAAG	C
Antibg29	TTTGAAAGCATTTTCGGGAC CTGTACACTCTGCCGGTCAA	C
Antibg30	TCCTTTCATTCCTCTCCATCA TTTGGAGCCACCTTCATTTC	C
Antibg33	CAAATGACATCCAAAAGATAATACAA AGAGATTTAGGCGATACAAGCA	C
Antibg39	ATACTGGGACCCACAAGCTG CTTCACCAAACCGCAAGATT	C

removed markers) as implemented in GENODIVE v2.0. We also estimated global Fst amongst patches. Genetic differentiation was also measured per year to investigate temporal variation in Fst. Significance of probabilities for Fst values were estimated using permutations of alleles, either overall or between pairs of populations.

Parentage analysis and pedigree reconstruction

We ensured that the set of microsatellite markers that we used was highly reliable for parentage analysis by estimating Parent-Pair exclusion probability (PPexp, Wang, 2007). PPexp for each marker and cumulative PPexp were estimated with the FRANz software (Riester *et al.*, 2009; Riester *et al.*, 2010). Individuals were assigned to parental genotypes using a Bayesian pedigree reconstruction approach that takes into account uncertainty about age; with age being estimated by the software on the basis the first year of sampling of each plant. We performed the parentage analysis in FRANz with the default parameter settings, except for: maximum number of candidate fathers (Nmax=14000), age range in which females and males can reproduce (femrepro=0:20; malerepro=0:20) to largely cover their lifespan, minimum number of typed loci (mintyped=16), convergence tolerance (saepsilon=0.1) and increment in the steady states distribution variation (sadelta=0.01) (see Almudevar, 2003 for more details on parameterisation).

We only included in the final pedigree the parentage assignments with a posterior probability higher than 0.95, which indicates that this parentage link was found in at least 95 of the 100 potential pedigrees reconstructed by using the assignment approach presented above. We only kept individuals with two reliably assigned parents (triads) to avoid false assignments arising from overlapping generations when using dyads. Each triad (offspring, parent1 & parent2) is also characterised by location information (patches from 1 to 6), GNSS coordinates, and their first year of sampling. We produced descriptive pedigree statistics (e.g. number of parent-offspring relationships, number of full sibling links, etc.) by using the Sequoia (v2.1.3, updates for hermaphrodites, Huisman, 2017) and Pedantics (Morrissey & Wilson, 2010) R packages. The type of family relationship between individuals were identified with the function GetRelCat of the Sequoia package. Individuals were then assigned to families to identify the different types of families, the number of family members, and their spread across generations with the package kinship2 (Sinnwell *et al.*, 2014) (makefamid function).

Functional connectivity and geographical distance among patches

Based on the parentage assignment and the location of plants, we estimated the number of within-patch dispersal events (i.e., when parents and offspring are found in the same patch), and between-patch dispersal events (i.e., when at least one parent is found on a different patch than the offspring). Dispersal distance was estimated by the geographical Euclidean distance in meters between parents and offspring using the R package Raster (Hijmans *et al.*, 2021). The closest parent (P1) was consistently located in the same patch than the offspring, which was expected because seeds are dispersed by gravity. Functional connectivity was estimated as the ratio of the total number of between-patch dispersal events and the total number of effective dispersal events overall patches. We also estimated per patch, outbound connectivity as the percentage of offspring on a different patch with the farthest parent on a given focal patch, inbound connectivity as the percentage of offspring on the given focal patch with the farthest parent on a different patch and total connectivity per patch as the sum of inbound and outbound connectivity. Finally, we tested whether patch-pairwise functional connectivity – estimated as the sum of effective dispersal events between two patches divided by the sum of effective dispersal events of these patches multiplied by 100 – was correlated with between-patch geographical distance by using a Spearman correlation test.

Reproductive success

To assess if functional connectivity was effective, we used the multigenerational pedigree to calculate the reproductive success estimated by the number of offspring of each plant resulting from a between-patch dispersal event, and each plant with resident parents. We removed plants sampled during the last two years of the survey (2018 and 2019) to avoid a temporal bias because the probability of finding their offspring in the field was weak. We assessed if the reproductive success was different between plants resulting from between-patch dispersal events, and plants with resident parents. To this aim, we built a negative binomial linear mixed model with a logit function to linearize the reproductive success count data, which accounts for count data overdispersion. We included the "dispersal status" of parents as a fixed effect (0 for resident parents and 1 for parents from different patches). We also included in the model the random effects on the intercept of the identity of patches and the closest and farthest parent to account for the non-independence of observations due to their location and genotype. This model was computed with the glmmTMB package (Magnusson *et al.*, 2021). We checked that the necessary assumptions of the model were respected (e.g., uniformity, overdispersion, outliers) with the DHARMA package (Hartig & Lohse, 2021). All analyses have been conducted with R version 3.6.3 (R Core Team, 2020).

Data availability

Underlying data

Zenodo: Code and data for "Wild snapdragon plant pedigree sheds light on limited connectivity enhanced by higher migrant reproductive success in a fragmented landscape",

https://doi.org/10.5281/zenodo.5682659 (Gervais *et al.*, 2021)

This project contains the following underlying data:

- freq_MCMC_1
- locisummary_MCMC_1.txt
- mcmc_MCMC_1.log
- mismatch_MCMC_1.txt
- output.txt
- parentage_MCMC_1.csv

- pedigree_MCMC_1.dat
- pedigree_MCMC_1.txt
- simulation_MCMC_1.txt
- summary_MCMC_1.txt
- geno_20102019.dat
- pedigree&location_metadata.txt
- pedigree_tryads_P095.Rdata

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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References

Aguilar R, Cristóbal-Pérez EJ, Balvino-Olvera FJ, *et al.*: **Habitat fragmentation reduces plant progeny quality: A global synthesis.** *Ecol Lett.* 2019; **22**(7): 1163–1173.
PubMed Abstract | **Publisher Full Text**

Aguilar R, Quesada M, Ashworth L, *et al.*: **Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches.** *Mol Ecol.* 2008; **17**(24): 5177–5188.
PubMed Abstract | **Publisher Full Text**

Almudevar A: **A simulated annealing algorithm for maximum likelihood pedigree reconstruction.** *Theor Popul Biol.* 2003; **63**(2): 63–75.
PubMed Abstract | **Publisher Full Text**

Andalo C, Cruzan MB, Cazettes C, *et al.*: **Post-pollination barriers do not explain the persistence of two distinct *Antirrhinum* subspecies with parapatric distribution.** *Plant Syst Evol.* 2010; **286**(3): 223–234.
Publisher Full Text

Auffret AG, Rico Y, Bullock JM, *et al.*: **Plant functional connectivity – integrating landscape structure and effective dispersal.** *J Ecol.* 2017; **105**(6): 1648–1656.
Publisher Full Text

Bacles CFE, Ennos RA: **Paternity analysis of pollen-mediated gene flow for *Fraxinus excelsior* L. in a chronically fragmented landscape.** *Heredity (Edinb).* 2008; **101**(4): 368–380.
PubMed Abstract | **Publisher Full Text**

Bacles CFE, Lowe AJ, Ennos RA: **Effective Seed Dispersal Across a Fragmented Landscape.** *Science.* 2006; **311**(5761): 628.
PubMed Abstract | **Publisher Full Text**

Bancroft JS, Turchin P: **An Experimental Test of Fragmentation and Loss of Habitat with Oryzaephilus Surinamensis.** *Ecology.* 2003; **84**(7): 1756–1767.
Publisher Full Text

Bonte D, De Roissart A, Wybouw N, *et al.*: **Fitness maximization by dispersal: Evidence from an invasion experiment.** *Ecology.* 2014; **95**(11): 3104–3111.
Publisher Full Text

Cayuela H, Rougemont Q, Prunier JG, *et al.*: **Demographic and genetic approaches to study dispersal in wild animal populations: A methodological review.** *Mol Ecol.* 2018; **27**(20): 3976–4010.
PubMed Abstract | **Publisher Full Text**

Chapman RE, Wang J, Bourke AFG: **Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators.** *Mol Ecol.* 2003; **12**(10): 2801–2808.
PubMed Abstract | **Publisher Full Text**

Dauber J, Bengtsson J, Lenoir L: **Evaluating effects of habitat loss and land-use continuity on ant species richness in seminatural grassland remnants.** *Conserv Biol.* 2006; **20**(4): 1150–1160.
PubMed Abstract | **Publisher Full Text**

Debout GDG, Lhuillier E, Malé PJG, *et al.*: **Development and characterization of 24 polymorphic microsatellite loci in two *Antirrhinum majus* subspecies (Plantaginaceae) using pyrosequencing technology.** *Conserv Genet Resour.* 2012; **4**(1): 75–79.
Publisher Full Text

Dick CW, Etchelecu G, Austerlitz F: **Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest.** *Mol Ecol.* 2003; **12**(3): 753–764.
PubMed Abstract | **Publisher Full Text**

Dupont A: **L'exploitation du sel sur les étangs de Languedoc (IXe-XIIIe siècle).** *Annales du Midi.* 1958; **70**(41): 7–25.
Publisher Full Text

Excoffier L, Foll M, Petit RJ: **Genetic Consequences of Range Expansions.** *Annu Rev Ecol Evol Syst.* 2009; **40**(1): 481–501.
Publisher Full Text

Fahrig L: **Ecological responses to habitat fragmentation per se.** *Annu Rev Ecol Evol Syst.* 2017; **48**: 1–23.
Publisher Full Text

Fahrig L, Arroyo-Rodríguez V, Bennett JR, *et al.*: **Is habitat fragmentation bad for biodiversity?** *Biol Conserv.* 2019; **230**: 179–186.
Publisher Full Text

Fahrig L, Merriam G: **Conservation of Fragmented Populations.** *Conserv Biol.* 1994; **8**(1): 50–59.
Publisher Full Text

Frankham R: **CONSERVATION GENETICS.** *Annu Rev Genet.* 1995; **29**: 305–27.
PubMed Abstract | **Publisher Full Text**

Galpern P, Manseau M, Hettinga P, *et al.*: **Allelematch: An R package for identifying unique multilocus genotypes where genotyping error and missing data may be present.** *Mol Ecol Resour.* 2012; **12**(4): 771–778.
PubMed Abstract | **Publisher Full Text**

Gervais L, Mouginot P, Gibert A, *et al.*: **Code and data for "Wild snapdragon plant pedigree sheds light on limited connectivity enhanced by higher migrant reproductive success in a fragmented landscape".** [Data set]. *Zenodo.* 2021.
http://www.doi.org/10.5281/zenodo.5682659

Gittleman JL, Gosling ML, Woodroffe R, *et al.*: **Genetics, demography and viability of fragmented populations.** Cambridge University Press. 2000.

Goverde M, Schweizer K, Baur B, *et al.*: **Small-scale habitat fragmentation effects on pollinator behaviour: Experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands.** *Biol Conserv.* 2002; **104**(3): 293–299.
Publisher Full Text

Hartig F, Lohse L: **DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.** (0.4.3) [Computer software]. 2021.
Reference Source

Hebel I, Aldinger E, Haas R, *et al.*: **Pollen dispersal and pollen contamination in an ash seed orchard (Fraxinus excelsior L.).** *Allgemeine Forst Und Jagdzeitung.* 2007; **178**(2–3): 44–49.
Reference Source

Hijmans RJ, van EJ, Sumner M, *et al.*: **raster: Geographic Data Analysis and Modeling.** (3.4-13) [Computer software]. 2021.
Reference Source

Huisman J: **Pedigree reconstruction from SNP data: Parentage assignment, sibship clustering and beyond.** *Mol Ecol Resour.* 2017; **17**(5): 1009–1024.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Kamm U, Gugerli F, Rotach P, *et al.*: **Open areas in a landscape enhance pollen-mediated gene flow of a tree species: Evidence from northern Switzerland.** *Landsc Ecol.* 2010; **25**(6): 903–911.
Publisher Full Text

Khimoun A, Burrus M, Andalo C, *et al.*: **Locally asymmetric introgressions between subspecies suggest circular range expansion at the *Antirrhinum majus* global scale.** *J Evol Biol.* 2011; **24**(7): 1433–1441.
PubMed Abstract | **Publisher Full Text**

Khimoun A, Cornuault J, Burrus M, *et al.*: **Ecology predicts parapatric distributions in two closely related *Antirrhinum majus* subspecies.** *Evol Ecol.* 2013; **27**(1): 51–64.
Publisher Full Text

Kunin WE: **Population Size and Density Effects in Pollination: Pollinator**

Foraging and Plant Reproductive Success in Experimental Arrays of Brassica Kaber. *J Ecol.* 1997; **85**(2): 225–234.
Publisher Full Text

Larguier G: **Chapitre VII. La mer, la terre, le sel une croissance narbonnaise au xvie s.** In: *Le drap et le grain en Languedoc: Narbonne et Narbonnais 1300-1789*. Presses universitaires de Perpignan, 2014; 408–490.
Reference Source

Lowe WH, Allendorf FW: **What can genetics tell us about population connectivity?** *Mol Ecol.* 2010; **19**(15): 3038–3051.
PubMed Abstract | **Publisher Full Text**

Magnusson A, Skaug H, Nielsen A, *et al.*: **glmmTMB: Generalized Linear Mixed Models using Template Model Builder.** (1.1.2) [Computer software]. 2021.
Reference Source

Meirmans PG, van Tienderen PH: **genotype and genodive: Two programs for the analysis of genetic diversity of asexual organisms.** *Mol Ecol Notes.* 2004; **4**(4): 792–794.
Publisher Full Text

Mills LS, Allendorf FW: **The One-Migrant-per-Generation Rule in Conservation and Management.** *Conserv Biol.* 1996; **10**(6): 1509–518.
Publisher Full Text

Moran EV, Clark JS: **Estimating seed and pollen movement in a monoecious plant: A hierarchical Bayesian approach integrating genetic and ecological data.** *Mol Ecol.* 2011; **20**(6): 1248–1262.
PubMed Abstract | **Publisher Full Text**

Morrissey MB, Wilson AJ: **pedantics: an r package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing.** COMPUTER PROGRAM ARTICLE. *Mol Ecol Resour.* 2010; **10**(4): 711–719.
PubMed Abstract | **Publisher Full Text**

Mueller T, Lenz J, Caprano T, *et al.*: **Large frugivorous birds facilitate functional connectivity of fragmented landscapes.** *J Appl Ecol.* 2014; **51**(3): 684–692.
Publisher Full Text

Nason JD, Hamrick JL: **Reproductive and Genetic Consequences of Forest Fragmentation: Two Case Studies of Neotropical Canopy Trees.** *J Hered.* 1997; **88**(4): 264–276.
Publisher Full Text

Pradel R: **Utilization of Capture-Mark-Recapture for the Study of Recruitment and Population Growth Rate.** *Biometrics.* 1996; **52**(2): 703–709.
Publisher Full Text

Pujol B, Archambeau J, Bontemps A, *et al.*: **Mountain landscape connectivity and subspecies appurtenance shape genetic differentiation in natural plant populations of the snapdragon (*Antirrhinum majus* L.).** *Bot Lett.* 2017; **164**(2): 111–119.
Publisher Full Text

R Core Team: **R: A Language and Environment for Statistical Computing**. R Foundation for Statistical Computing. 2020.
Reference Source

Riester M, Stadler PF, Klemm K: **FRANz: Reconstruction of wild multi-generation pedigrees.** *Bioinformatics.* 2009; **25**(16): 2134–2139.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Riester M, Stadler PF, Klemm K: **Reconstruction of pedigrees in clonal plant populations.** *Theor Popul Biol.* 2010; **78**(2): 109–117.
PubMed Abstract | **Publisher Full Text**

Ringbauer H, Kolesnikov A, Field DL, *et al.*: **Estimating Barriers to Gene Flow from Distorted Isolation-by-Distance Patterns.** *Genetics.* 2018; **208**(3): 1231–1245.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Robertson EP, Fletcher RJ Jr, Austin JD: **The number of breeders explains genetic connectivity in an endangered bird.** *Mol Ecol.* 2019; **28**(11): 2746–2756.
PubMed Abstract | **Publisher Full Text**

Robertson EP, Fletcher RJ Jr, Cattau CE, *et al.*: **Isolating the roles of movement and reproduction on effective connectivity alters conservation priorities for an endangered bird.** *Proc Natl Acad Sci U S A.* 2018; **115**(34): 8591–8596.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Ronce O: **How Does It Feel to Be Like a Rolling Stone? Ten Questions About Dispersal Evolution.** *Annu Rev Ecol Evol Syst.* 2007; **38**(1): 231–253.
Publisher Full Text

Schupp EW, Jordano P, Gómez JM: **Seed dispersal effectiveness revisited: A conceptual review.** *New Phytol.* 2010; **188**(2): 333–353.
PubMed Abstract | **Publisher Full Text**

Sebbenn AM, Carvalho ACM, Freitas MLM, *et al.*: **Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf.** *Heredity (Edinb).* 2011; **106**(1): 134–145.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Sinnwell JP, Therneau TM, Schaid DJ: **The kinship2 R Package for Pedigree Data.** *Hum Hered.* 2014; **78**(2): 91–93.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Sork VL, Nason J, Campbell DR, *et al.*: **Landscape approaches to historical and contemporary gene flow in plants.** *Trends Ecol Evol.* 1999; **14**(6): 219–224.
PubMed Abstract | **Publisher Full Text**

Tastard E, Ferdy JB, Burrus M, *et al.*: **Patterns of floral colour neighbourhood and their effects on female reproductive success in an *Antirrhinum* hybrid zone.** *J Evol Biol.* 2012; **25**(2): 388–399.
PubMed Abstract | **Publisher Full Text**

Tischendorf L, Fahrig L: **On the usage and measurement of landscape connectivity.** *Oikos.* 2000; **90**(1): 7–19.
Publisher Full Text

Uroy L, Ernoult A, Mony C: **Effect of landscape connectivity on plant communities: A review of response patterns.** *Landsc Ecol.* 2019; **34**(2): 203–225.
Publisher Full Text

Vasudev D, Fletcher RJ Jr: **Mate choice interacts with movement limitations to influence effective dispersal.** *Ecol Model.* 2016; **327**: 65–73.
Publisher Full Text

Vittoz P, Engler R: **Seed dispersal distances: A typology based on dispersal modes and plant traits.** *Bot Helv.* 2007; **117**(2): 109–124.
Publisher Full Text

Vranckx G, Jacquemyn H, Muys B, *et al.*: **Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation.** *Conserv Biol.* 2012; **26**(2): 228–237.
PubMed Abstract | **Publisher Full Text**

Wade AA, Hand BK, Kovach RP, *et al.*: **Accounting for adaptive capacity and uncertainty in assessments of species' climate-change vulnerability.** *Conserv Biol.* 2017; **31**(1): 136–149.
PubMed Abstract | **Publisher Full Text**

Walther-Hellwig K, Frankl R: **Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape.** *J Appl Entomol.* 2000; **124**(7–8): 299–306.
Publisher Full Text

Wang J: **Parentage and sibship exclusions: Higher statistical power with more family members.** *Heredity (Edinb).* 2007; **99**(2): 205–217.
PubMed Abstract | **Publisher Full Text**

Wang J, Kang M, Gao P, *et al.*: **Contemporary pollen flow and mating patterns of a subtropical canopy tree *Eurycorymbus cavaleriei* in a fragmented agricultural landscape.** *For Ecol Manage.* 2010; **260**(12): 2180–2188.
Publisher Full Text

Whitlock MC, McCauley DE: **Indirect measures of gene flow and migration: FST not equal to 1/(4Nm + 1).** *Heredity (Edinb).* 1999; **82**(pt 2): 2.
PubMed Abstract | **Publisher Full Text**

Williams JL, Hufbauer RA, Miller TEX: **How Evolution Modifies the Variability of Range Expansion.** *Trends Ecol Evol.* 2019; **34**(10): 903–913.
PubMed Abstract | **Publisher Full Text**

Zurbuchen A, Landert L, Klaiber J, *et al.*: **Maximum foraging ranges in solitary bees: Only few individuals have the capability to cover long foraging distances.** *Biol Conserv.* 2010; **143**(3): 669–676.
Publisher Full Text

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✔ **Yann X. C. Bourgeois**
School of Biological Sciences, University of Portsmouth, Portsmouth, UK

The authors have answered all my comments. I understand that some aspects may be further investigated in future studies. I do not have any major concerns about this work.
All the best.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am an evoutionary biologist interested in population genetics and genomics, with a focus on fast adaptation and transposable elements.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 13 January 2023

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✔ **Josselin Clo**
Charles University, Prague, Czech Republic

I am happy with the authors' responses and modifications to the manuscript.

I found the text much clearer after taking into account the reviewers' comments, and I am still thinking it is a nice article and contribution to the field.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am an evolutionary biologist exploring how key life-history traits (inbreeding and polyploidy) affect the evolutionary potential of populations.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 07 July 2022

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Yann X. C. Bourgeois
School of Biological Sciences, University of Portsmouth, Portsmouth, UK

In this work, the authors contrast functional and effective connectivity at a small spatial scale in a population of wild snapdragons divided into six distinct patches. They conducted a demographic survey over several years and estimated migration rates between isolated patches to test whether higher reproductive success of migrants may counterbalance genetic isolation. They show that despite barriers to dispersal between patches, the higher fitness of individuals with parents from different patches may compensate for their relative rarity and rescue populations. The analyses are sound and the amount of fieldwork this study represents is impressive. The manuscript is well written.

After reading the Abstract and Introduction, and references therein, I was left with the impression that the authors think that populations may **benefit** from a fragmented habitat here. While it is clear that populations do not go extinct, they may still be growing at a lower rate than more connected populations. Instead of benefitting populations, fragmentation increases inbreeding within patches, lowering genetic diversity, while migration rescues local patches, which can keep growing. Without a proper comparison of this population with another large, non-fragmented one, it remains difficult to draw any clear conclusion about the meaning of positive growth rates. I feel like this should be more clearly stated.

Comments about functional connectivity estimates:

- Pedigrees are useful, but relying solely on them leaves the door open to ascertainment biases due to missing parents/offsprings or unassigned individuals. This means that the “2.9% of offspring [that] had one parent on a different patch” is probably an underestimate of the actual connectivity. Ghost (unsampled) populations may also contribute to the

homogenization of gene pools, and it would be worth presenting more clearly how many individuals had one or two unidentified parents in each patch. I would not be surprised if that number was higher in the patches bordering the study area (i.e. 1 and 6).

- I do not agree that functional connectivity is “weak”. FST are significant but low (ranging from 0.01 to 0.08 at most). Using the Wright’s equation as a rule of thumb (FST ≈ 1/(4Nem + 1)), this suggests a number of migrant alleles between patches that is at the very least around 3 per generation. This is higher than the classical "One migrant per generation" rule used to determine whether populations are drifting apart. This warrants a more nuanced discussion throughout the paper.

- Other methods may also be used to estimate relatedness for all pairs of geolocated individuals, between and within patches. The authors could then assess whether there is any significant barrier to gene flow. One example of spatially-explicit method that can handle microsatellites is EEMS (Petkova, Novembre, & Stephens, 2015[1]). It might be worth applying it here to examine whether the diffusion of alleles is significantly higher between patches than within them. Such analyses could include the vast majority of individuals not included in the pedigree.

Comments on effective connectivity (migrant reproductive success):

- There are many well documented examples showing an advantage for migration that restores genetic diversity and, e.g., limits the exposure of deleterious alleles at the homozygous state. One of the most interesting aspects of the study in my opinion is the quantitative estimate of migrant reproductive success. It may be worth providing an estimate of individual inbreeding to test for a correlation between reproductive success and heterozygosity for example. One could also compare populations growth rates with the number of migrants detected every year, or with average FST.

Miscellaneous:

- Table 3: I assume these statistics are produced by pooling all individuals. I would recommend an analysis per year and per population. Deviations from Hardy-Weinberg equilibrium may reveal strong local inbreeding for example. In addition, the lack of deviation from Hardy-Weinberg equilibrium suggests that Wahlund effects due to underlying population structure are not pronounced. Some authors would even consider this as a lack of evidence for any significant population structure (cf comment above about the “weak” connectivity).

I hope you find these comments constructive.

References

1. Petkova D, Novembre J, Stephens M: Visualizing spatial population structure with estimated effective migration surfaces.*Nat Genet*. 2016; **48** (1): 94-100 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and does the work have academic merit?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am an evoutionary biologist interested in population genetics and genomics, with a focus on fast adaptation and transposable elements.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 14 Dec 2022

Laura Gervais

We thank you for your comments that helped improving this paper and gave us perspective for future research questions on this study system. You will find our point by point response preceded by “>>Authors response". All line numbers refer to the updated manuscript file containing changes highlighted in yellow. Please note that the track-changed manuscript is available on zenodo here https://doi.org/10.5281/zenodo.7438502. ----------
Yann X. C. Bourgeois, School of Biological Sciences, University of Portsmouth, Portsmouth, UK

In this work, the authors contrast functional and effective connectivity at a small spatial scale in a population of wild snapdragons divided into six distinct patches. They conducted a demographic survey over several years and estimated migration rates between isolated patches to test whether higher reproductive success of migrants may counterbalance genetic isolation. They show that despite barriers to dispersal between patches, the higher fitness of individuals with parents from different patches may compensate for their relative rarity and rescue populations. The analyses are sound and the amount of fieldwork this study represents is impressive. The manuscript is well written. After reading the Abstract and Introduction, and references therein, I was left with the impression that the authors think that populations may benefit from a fragmented habitat here. While it is clear that populations do not go extinct, they may still be growing at a lower rate than more connected populations. Instead of benefitting populations, fragmentation increases inbreeding within patches, lowering genetic diversity, while migration rescues local patches, which can keep growing. Without a proper comparison of this population with another

large, non-fragmented one, it remains difficult to draw any clear conclusion about the meaning of positive growth rates. I feel like this should be more clearly stated.

>> Author response: We thank the reviewer for pointing this issue which was not our intention. We clarified the text throughout the paper, and in particular in the introduction, to outline that we are aware of the negative impact of fragmentation, and that we do not think snapdragon plant populations benefit directly from fragmentation. We agree with you. Our aim is to outline that based on the literature, mechanisms at play in populations that are surviving and expanding in fragmented landscapes are unclear. We do not intend to interpret positive growth rates as a property of fragmented versus non-fragmented large populations. We simply present here an example of limited functional connectivity, which role played against reproductive isolation may have been underestimated if we had not found out that the few propagule exchanges were efficient as they were associated with higher reproductive success.

Comments about functional connectivity estimates:

- Pedigrees are useful, but relying solely on them leaves the door open to ascertainment biases due to missing parents/offsprings or unassigned individuals. This means that the “2.9% of offspring [that] had one parent on a different patch” is probably an underestimate of the actual connectivity. Ghost (unsampled) populations may also contribute to the homogenization of gene pools, and it would be worth presenting more clearly how many individuals had one or two unidentified parents in each patch. I would not be surprised if that number was higher in the patches bordering the study area (i.e. 1 and 6).

>> Author response: We agree that true estimates of connectivity can only be higher than estimates obtained from pedigrees because of the presence of ghost parents. This is a widely acknowledged limitation of all pedigree-based connectivity studies. There are two ways to overcome at least partly this issue: 1. focus on robust “family triangles” (two parents + offspring) across patches that were exhaustively surveyed (with of course a few missing samples). 2. use geographic sites which natural setting is similar to an optimal experimental design (eg. when 6 patches of plants are located on islands, or 3 lagoons around an island are far from the main coast). As a result, estimated connectivity can be used to infer migration processes between surveyed patches because a large sample of plants is used but these connectivity estimates cannot be used to infer what is ongoing beyond this geographic scale. Here, the pedigree analysis allowed us to identify directional trends in connectivity, which allowed us to identify the plants directly involved in effective connectivity events between the 6 sites that are surveyed. We had already mentioned in the text that the presence of ghost parents "suggests migration from outside the studied area” the text. We accommodated your comment in the revised text by presenting numbers for unidentified parents L111, L125-128.

- I do not agree that functional connectivity is “weak”. FST are significant but low (ranging from 0.01 to 0.08 at most). Using the Wright’s equation as a rule of thumb ($F_{ST} \approx 1/(4Nem + 1)$), this suggests a number of migrant alleles between patches that is at the very least around 3 per generation. This is higher than the classical "One migrant per generation" rule used to determine whether populations are drifting apart. This warrants a more nuanced discussion throughout the paper.

>> Author response: We agree with the reviewer that with a few migrants per generation

(not far from the “one migrant per generation rule”), connectivity plays an important role. This is empirically supported by our estimates of connectivity, and this is our take home message. We now mention more clearly this aspect in the text and present scenarii of migration counterbalancing drift rooted in our coherent FST and connectivity results L185-190; L195-196. We also removed “weak connectivity” from the text and nuanced the discussion of this aspect throughout the paper.

- Other methods may also be used to estimate relatedness for all pairs of geolocated individuals, between and within patches. The authors could then assess whether there is any significant barrier to gene flow. One example of spatially-explicit method that can handle microsatellites is EEMS (Petkova, Novembre, & Stephens, 20151). It might be worth applying it here to examine whether the diffusion of alleles is significantly higher between patches than within them. Such analyses could include the vast majority of individuals not included in the pedigree.

>> Author response: We agree that other relatedness methods using all pairs of individuals can also be used to infer spatial patterns of effective migration. Here, the pedigree analysis allowed us to identify directional trends in connectivity, notably based on robust “family triangles” (two parents + offspring), which allowed us to identify the plants directly involved in effective connectivity events between the 6 sites that are surveyed. This pedigree approach has the advantage of estimating both migration events and reproductive success. As outlined in the text, we want to encourage studies estimating connectivity to also infer reproductive success which is for now, to our knowledge, only possible with a pedigree approach. We thank you for outlining the interest of using the EEMS approach and are enthusiastic about using it in another study more specifically aiming at evaluating the role of ecological barriers.

Comments on effective connectivity (migrant reproductive success):

- There are many well documented examples showing an advantage for migration that restores genetic diversity and, e.g., limits the exposure of deleterious alleles at the homozygous state. One of the most interesting aspects of the study in my opinion is the quantitative estimate of migrant reproductive success. It may be worth providing an estimate of individual inbreeding to test for a correlation between reproductive success and heterozygosity for example.

>> Author response: We agree that the link between migration and a higher reproductive success is a key finding of our study and that research aiming at understanding the underlying mechanisms is very promising. We also agree that HFC (heterozygote-fitness correlations) could be used to investigate the link between inbreeding and reproductive success, which may be itself linked to migration. However, many ecological or genetic mechanisms may be at play here. An investigation of the inbreeding-fitness correlation would be in itself a demanding study requiring to explore many evolutionary scenarii. As such, it would be difficult to include this aspect in this paper without carrying the reader away from the take home message of this study. We thank the reviewer for outlining this research track that we will hope to follow in the near future.

- One could also compare populations growth rates with the number of migrants detected every year, or with average FST.

>> Author response: We agree that behind the test proposed by the reviewer lies a very interesting research question. However, we do not think that our dataset allows to reliably test for this hypothesis. There is likely a time lag between the current arrival of migrants

and their participation to the population growth, and a temporal disconnection with genetic differentiation. We also think that average FST estimates may not be very well fitted for this type of statistical analysis. We took this comment into account by outlining in the revised text that it would be interesting to investigate the link between the growth rate and differentiation (or in fact connectivity) but did not go further in the details to avoid speculation L227-228; L230-232.

Miscellaneous:

- Table 3: I assume these statistics are produced by pooling all individuals. I would recommend an analysis per year and per population.Deviations from Hardy-Weinberg equilibrium may reveal strong local inbreeding for example. In addition, the lack of deviation from Hardy-Weinberg equilibrium suggests that Wahlund effects due to underlying population structure are not pronounced. Some authors would even consider this as a lack of evidence for any significant population structure (cf comment above about the “weak” connectivity).

>> Author response: Indeed, the statistics of table 3 are made by pooling individuals among years and patches. We added this information in the legend of table 3 L86-87. In the genetic differentiation analysis, we only used markers that were at HW equilibrium as required to respect statistical assumptions. We added this information in the methods section L306-308. Since FST was very low, there is little opportunity for Wahlund effects. Using deviation from HW equilibrium to reveal the lack of between-population differentiation in these snapdragon patches would be unreliable. I hope you find these comments constructive.

Competing Interests: No competing interests were disclosed.

Reviewer Report 21 February 2022

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Josselin Clo
Charles University, Prague, Czech Republic

I had the pleasure to read and review the manuscript “Wild snapdragon plant pedigree shed lights on limited connectivity enhanced by higher migrant reproductive success in a fragmented landscape”, from Gervais *et al*. The aim of the manuscript is to investigate the Snapdragon demographic dynamic during several years, in a population fragmented in several isolated patches. They associated the demographic survey with an analysis of the effective connectivity between patches in order to understand the demographic results. The main results of the study are that all the patches within the populations are increasing their demographic sizes, despite very low gene flows among patches. Nevertheless, if the genetic exchanges are rare, the fitness of

migrant plants is higher than the one of local plants, potentially explaining the demographic patterns.

Overall, the manuscript is well-written. The dataset used is impressive, with a sampling of more than 12.000 plants over ten years. The introduction prepares the reader for what will be presented and discussed later in the manuscript. It offers a quick but comprehensive summary of the effect of habitat fragmentation on the evolutionary potential of the (sub-)populations, and how the (effective) connectivity between fragmented habitats can help the populations in overcoming the above-mentioned negative effects. The other sections are also overall well written, in a concise style which is nice to read. The results answered the questions presented in the introduction. The results are not overstated. In addition, the data and Rcode used are already available in public repositories.

I have only one major comment that I would like to discuss with the authors, and several "cosmetic" minor comments, that I hope will help the authors in increasing a little bit more the quality and clarity of the manuscript.

Major comment:

The authors stated that the observed population expansion is due to the higher reproductive success after migration, which compensates for the other deleterious effects of habitat fragmentation. It could be true, but as presented, it is not the only possible explanation. The high number of sampling plants suggests that the population of Snapdragon was already well established in this former industrial location, it is possible that the population is just well adapted to its environment, and that even without the help of the fittest migrants the sub-populations would be in a demographic expansion?
I think that you could try to correlate the growth rates of patches with your measures of connectivity (the functional and effective connectivity), to see if higher growth rates are associated with more fittest migrants arriving in the patches?

Minor comments:

The formatting of the manuscript avoids giving the line numbers. I tried to be as clear as possible for you to find the location of the comment, by advance sorry if it is not clear.

Introduction:

First paragraph: the authors can also mention the Wood *et al.* (2016[1]) meta-analysis showing that smaller demographic population sizes are not associated with a decrease in adaptive potential.

Results:

Demographic expansion:
Table 2: The legend indicates that a growth rate higher than 1 should be in bold, but this is not the case. Either modify the legend or the table. Some growth rates are incredibly high (for example patch 1 in 2015), is it a real growth rate, or did you forget the brackets?

Genetic diversity:

The material and method section being after the result section, some parameters and their abbreviations are not described. Please indicate what is Hs the first time you mention it, and what represents the variation around the mean value (95% confidence interval, standard deviation?), and indicate it also in the M&M section.

Low functional connectivity between the six patches:
Figure 4: The visual quality of figure 4 is low compared to the three others, is it possible to improve it?

Discussion:

First paragraph: Please propose alternative scenarios.

Higher reproductive success associated with connectivity:
Please give more details of why migrant plants can have higher fitness than the local ones.

Material and methods:

GPS coordinates: Most of the points were recorded with the GNSS receiver, but how were the few hundred remaining points recorded?
Statistical tests: Several p-values are reported in the “Results section” (for Fst estimations for example), but the statistical tests are not described in the material and method section. Please indicate the test you performed through the whole M&M section.
Parentage analysis: Please indicate the default values of other parameters, if there are not too many.

References
1. Wood JL, Yates MC, Fraser DJ: Are heritability and selection related to population size in nature? Meta-analysis and conservation implications.*Evol Appl*. **9** (5): 640-57 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am an evolutionary biologist exploring how key life-history traits (inbreeding and polyploidy) affect the evolutionary potential of populations.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 14 Dec 2022

Laura Gervais

We would like to thank the reviewer for his positive feedbacks and constructive comments. You will find our point by point response preceded by ">>Authors response". All line numbers refer to the updated manuscript file containing changes highlighted in yellow. Please note that the track-changed manuscript is available on zenodo here https://doi.org/10.5281/zenodo.7438502.

I had the pleasure to read and review the manuscript "Wild snapdragon plant pedigree shed lights on limited connectivity enhanced by higher migrant reproductive success in a fragmented landscape", from Gervais et al. The aim of the manuscript is to investigate the Snapdragon demographic dynamic during several years, in a population fragmented in several isolated patches. They associated the demographic survey with an analysis of the effective connectivity between patches in order to understand the demographic results. The main results of the study are that all the patches within the populations are increasing their demographic sizes, despite very low gene flows among patches. Nevertheless, if the genetic exchanges are rare, the fitness of migrant plants is higher than the one of local plants, potentially explaining the demographic patterns. Overall, the manuscript is well-written. The dataset used is impressive, with a sampling of more than 12.000 plants over ten years. The introduction prepares the reader for what will be presented and discussed later in the manuscript. It offers a quick but comprehensive summary of the effect of habitat fragmentation on the evolutionary potential of the (sub-)populations, and how the (effective) connectivity between fragmented habitats can help the populations in overcoming the above-mentioned negative effects. The other sections are also overall well written, in a concise style which is nice to read. The results answered the questions presented in the introduction. The results are not overstated. In addition, the data and Rcode used are already available in public repositories. I have only one major comment that I would like to discuss with the authors, and several "cosmetic" minor comments, that I hope will help the authors in increasing a little bit more the quality and clarity of the manuscript. The authors stated that the observed population expansion is due to the higher reproductive success after migration, which compensates for the other deleterious effects of habitat fragmentation. It could be true, but as presented, it is not the only possible explanation. The high number of sampling plants suggests that the population of

Snapdragon was already well established in this former industrial location, it is possible that the population is just well adapted to its environment, and that even without the help of the fittest migrants the sub-populations would be in a demographic expansion?

>> Author response: We fully agree that one reason behind the expansion of these plants is likely that they are well adapted to their current environment. We did not wish to imply that expansion was caused by the higher reproductive success of migrants, and we did not wish to exclude other mechanisms. We only meant to imply that in a context of limited propagule exchanges (functional connectivity was low), a higher reproductive success after migration increases the chances that migrants participate to population dynamics and counteracts reproductive isolation between patches. We thank the reviewer for pointing this out. We have clarified this aspect throughout the text. I think that you could try to correlate the growth rates of patches with your measures of connectivity (the functional and effective connectivity), to see if higher growth rates are associated with more fittest migrants arriving in the patches?

>> Author response: Thank you for pointing out this interesting hypothesis. We included some text to mention that it would be interesting to investigate the link between the growth rate of patches and their connectivity L228-229. However, we do not think it is possible to test this hypothesis using our dataset L222-225. There is likely a time lag between the arrival of migrants and their participation to a given patch growth rate in wild snapdragon populations. We also think that it would be difficult to interpret a correlation between higher growth rates and the arrival of fitter migrants because this correlation would not imply causality in the wild because of multiple other drivers might be involved.

The formatting of the manuscript avoids giving the line numbers. I tried to be as clear as possible for you to find the location of the comment, by advance sorry if it is not clear.

>> Author response: Likewise. We had included line numbers but ORE removed them, which we think is deleterious to the review process for both authors and reviewers. We uploaded a revised version of the paper that includes line numbers to which we refer to in this response letter. If these numbers were again to be removed from the main document, know that we uploaded on the zenodo server a version named “track changes” that includes line numbers and where text modifications are outlined.

Introduction:

First paragraph: the authors can also mention the Wood et al. (20161) meta-analysis showing that smaller demographic population sizes are not associated with a decrease in adaptive potential.

>> Author response: We thank the reviewer for this reference. Yet we think this reference is not adequate for our paper. Wood et al showed no reduction of narrow-sense heritability with decreasing population size. Beyond the fact that choosing h^2 to compare the adaptive potential of populations from different contexts raises several issues, this study is not directly related to connectivity and fragmentation.

Results:

Demographic expansion:
Table 2: The legend indicates that a growth rate higher than 1 should be in bold, but this is not the case. Either modify the legend or the table. Some growth rates are incredibly high (for example patch 1 in 2015), is it a real growth rate, or did you forget the brackets?

>> Author response: Thanks for pointing this out. We clarified the legend and the contents of the table to outline numbers that are not growth rates but represent population sample sizes L65-69 . We did put population sample sizes between brackets to indicate when their short time fluctuations in patches characterized by small population sample sizes would result in abnormally high growth rates (e.g., from 1 to 50 plants over a year would lead to a growth rate value of 50). After discussing and revisiting the sampling data, we decided that patch 1 growth rates should be considered with extreme caution and specified this aspect in the text (Legend of Table 2 and methods, L67-69; L268-272). This is because the spatial heterogeneity of patch 1 might have compromised the possibility to replicate the exact same sampling area from one year to the next (notably in 2015). These short-term fluctuations do not challenge our conclusion about the global increase in population size. Nevertheless, we consider that these fluctuations limit our perspective on growth rates and exclude the possibility of using a correlative approach to analyse their variation beyond evaluating global trends. Genetic diversity:
The material and method section being after the result section, some parameters and their abbreviations are not described. Please indicate what is Hs the first time you mention it, and what represents the variation around the mean value (95% confidence interval, standard deviation?), and indicate it also in the M&M section.

>> Author response: Done L72, L299-300.

Low functional connectivity between the six patches:
Figure 4: The visual quality of figure 4 is low compared to the three others, is it possible to improve it?
>> Author response: Done.

Discussion:

First paragraph: Please propose alternative scenarios.

>> Author response: We followed the reviewer's suggestion and clarified the two scenarios that both rely on the balance between drift and migration. One scenario relies on the equilibrium of these forces, with a history of drift and likely low migration resulting in genetic differentiation and the other scenario involves a past history of divergence (linked to the same drifting mechanisms) but in the process of being resolved by connectivity. Connectivity might be recent and results at least partly from the demographic expansion that would have increased pollen flow. We did not enter into that much details in the text because we do not want to speculate too much. These two scenarii are now presented in the revised text L182-187. Higher reproductive success associated with connectivity:

Please give more details of why migrant plants can have higher fitness than the local ones.

>> Author response: We followed the reviewer's comment by adding more details L217. In terms of phenotypic performance, we now mention in the text the example from Williams et al 2016 who said "Plant height and dispersal ability evolved more rapidly in patchier experimental landscapes, suggesting that fragmentation can select for more rapid invasion velocity". We also added text about an alternative hypothesis, which is that the higher reproductive success of migrants can be explained by random processes (e.g. colonisation waves)L213-214. Obviously, we did not enter in too many details in the text of the paper because we do not want to speculate too much. In order to correctly investigate these mechanisms, it would be necessary to conduct a different type of study to test for these hypotheses. Material and methods:

GPS coordinates: Most of the points were recorded with the GNSS receiver, but how were the few hundred remaining points recorded?

>> Author response: Indeed 99 individuals did not have geographic coordinates, but have a sampling location noted as patch 1,2,3,4,5,6, which is sufficient for the connectivity measures. We add this information L263-264.

Statistical tests: Several p-values are reported in the "Results section" (for Fst estimations for example), but the statistical tests are not described in the material and method section. Please indicate the test you performed through the whole M&M section.

>> Author response: Done L307-308. Parentage analysis: Please indicate the default values of other parameters, if there are not too many.

>> Author response: There are over 40 different arguments in the FRANz function that are set to the default value. We think it is better to specify only those not set to default values in the text and invite readers to refer to the Franz manual on the Franz github repository to access all the default values https://github.com/lima1/franzpedigree.

Competing Interests: No competing interests were disclosed.