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Effects of human disturbance on reproductive success and population viability of *Serapias cordigera* (Orchidaceae)

GIUSEPPE PELLEGRINO* and FRANCESCA BELLUSCI

Department of Biology, Ecology and Earth Sciences, University of Calabria, I-87036 Rende (CS), Italy

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Fragmentation of habitats by roads, railroads, fields, buildings and other human activities can affect population size, pollination success and fruit production, especially in plants showing pollinator limitation, such as Mediterranean orchids. In this study, we investigated the effect of human activity on the population dynamics and reproductive success of the orchid *Serapias cordigera*. Three anthropic and three natural populations were monitored over 14 years (1999–2012), classifying individuals into five stage classes and evaluating reproductive success. Population growth rates differed between anthropic and natural populations. Our results demonstrated that small anthropic populations have lower population viability compared with large natural populations. The proportion of flowering plants, the number of reproductive plants and the percentage of fruits were significantly lower in anthropic than in natural populations. This strong decline in fruit production in populations in urban areas may reflect lower pollination attraction and higher inbreeding in small than in natural populations. Calculation of extinction probabilities showed that the anthropic populations will drop below the survival threshold of 15–20 years. This study highlights that continued monitoring is needed to improve information on population viability and for appropriate conservation management. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, **176**, 408–420.

ADDITIONAL KEYWORDS: anthropization – extinction – flowering – fruits – orchids – pollination.

INTRODUCTION

Although many plant populations are naturally isolated and small, populations of numerous plant species have become more isolated as a result of the recent anthropogenic fragmentation of habitats, which can alter the environment much more quickly than natural/ecological processes, contributing to the decline in population size of many plant species (Hobbs & Yates, 2003; Newman *et al*., 2013). Fragmentation of habitats by roads, railroads, fields, buildings and other human activities, increasing the spatial distance between plant populations, can hamper insect movement (Forman & Alexander, 1998; Öckinger, Dannestam & Smith, 2009), reduce pollinator abundance (Liu & Koptur, 2003) and alter their behaviour and the frequency of flower visits (Aguilar *et al*., 2006), thereby decreasing plant fitness (Peterson, Bartish & Peterson, 2008). Human-induced alterations to natural habitats often reduce the size and density of populations (McKinney, 2002; Aguilar *et al*., 2006), and the size and spatial structure of populations may affect the delivery of outcrossing pollen by influencing pollinator visitation and the availability of conspecific pollen donors (Hegland & Totland, 2012; Hanoteaux, Tielbörger & Seifan, 2013). In addition, the level of inbreeding may be higher in small, isolated populations (Miao *et al*., 2014) because of the higher rate of selfing and more frequent matings between close relatives. The resulting inbreeding depression can reduce the fitness of these plants compared with those in larger populations. Consequently, decreased outcrossing in small, sparse populations may reduce population fitness, potentially increasing the probability of extinction (Gargano *et al*., 2009; Stachurska-Swakon, Cieslak & Ronikier, 2011).

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*Corresponding author. E-mail: giuseppe.pellegrino@unical.it

Fruit set decrease as a result of reduction in insect movements is particularly strong for plants which show a high degree of dependence and specialization on their pollinator mutualism (i.e. pollinator limitation) for fruit production (Nayak & Davidar, 2010), such as Mediterranean deceptive orchids (Tremblay *et al*., 2005, and references therein). Sexual reproduction is predominantly pollinator dependent, even if it may sometimes be successfully guaranteed by asexual seed production (agamospermy) or by automatic self-pollination. In many non-autogamous orchids, fruit set is increased by hand pollination, suggesting that the observed variation in fruit production is mainly dependent upon the level of pollinator activity (Tremblay *et al*., 2005).

One of the main objectives of conservation biology is to predict the probability of population persistence based on estimates of reproduction and mortality. Several studies have shown that fragmented populations of orchids may be prone to extinction (Sletvold *et al*., 2010a) because of small population size, skewed lifespan and stochastic reproductive success (Willems, 2002; Tremblay & Hutchings, 2003).

Population viability analysis (PVA) entails the evaluation of data and models for a population to anticipate the likelihood that a population will persist for some arbitrarily chosen time into the future (Beissinger & McCullough, 2002; Gerber & González-Suárez, 2010). Fundamental to PVA is the fact that small populations are more likely to become extinct than larger ones (Matter & Roland, 2010) because of inbreeding depression, genetic drift or simply the threat of chance birth or death events (demographic stochasticity).

In the last decade, a large number of demographic studies have focused on orchids, with both short-term (Zotz, 1998; Winkler, Hülber & Hietz, 2009) and long-term (Zotz & Schimdt, 2006; Jacquemyn *et al*., 2007; Hutchings, 2010; Sletvold, Øien & Moen, 2010b; Sletvold *et al*., 2013) observations.

In this study, we investigated long-term dynamics to test the hypothesis that habitat fragmentation by human activity has a significant effect on reproductive success and on population viability by using a Mediterranean orchid species *Serapias cordigera* L. (Orchidaceae), collecting demographic data over 14 years (1999–2012) in permanent plots. This species forms large populations in the countryside, which contrast with small and 'patchy' populations in urban fragments, providing an ideal opportunity to examine how human activities affect orchid population dynamics and fruit set by comparing the number of specimens and reproductive success in anthropic populations with those in natural populations in the countryside of the same region. In this paper we address the following questions. Does habitat decay affect orchid population dynamics? Is fruit set related to increased isolation and fragmentation? Finally, a population viability analysis was performed to estimate extinction probabilities of the studied populations within specific time horizons.

MATERIAL AND METHODS

Study species

The genus *Serapias* L. is distributed throughout the Mediterranean region with its centre of diversity in southern Italy and on the Greek islands (Baumann & Künkele, 1989). A recent systematic treatment included 30 taxa (Delforge, 2006), which were characterized by a common floral morphology, with the sepals, petals and lateral lobes at the base of the lip forming a small tube. *Serapias cordigera* L. (heart-flowered *Serapias*) is a short-lived tuberous orchid; it has a three-lobed lip without a spur, two guiding swellings at the base of the lip and a plain-coloured epichile more than two-thirds the length of the hypochile, which confers a typically heart-shaped lip. Recent phylogenetic analysis strongly supports a natural split of *S. cordigera* into a subgroup also containing *Serapias nurrica* Corrias and *Serapias perez-chiscanoi* Acedo (Bellusci *et al*., 2008). The main pollinators are male bees of the genera *Eucera*, *Hoplitis*, *Osmia* and *Megachile* (N. J. Vereecken, pers. comm.) sleeping in the floral tube. The chromosome number is $2n = 36$ (D'Emerico, Pignone & Scrugli, 2000). It is a widespread species, mainly distributed in the western Mediterranean countries, but reaching southern Turkey and western North Africa; it grows in arid meadows, abandoned agricultural soils, garigue and bushy environments up to 900 m a.s.l. (Delforge, 2006). The species has declined in much of southern Europe and is endangered in its distribution range, probably as a result of habitat loss and habitat fragmentation by human activities.

Study sites and data collection

The present study examined population dynamic of *S. cordigera* at six populations located in the northern part of the Calabria region (southern Italy): three sites – Cutura (A1), Piano Monello (A2) and Piano Lago (A3) – are found in a highly anthropic landscape context enclosed by busy roads and their intersections, built in the late 1960s. The other three sites are non-anthropic (natural) populations in San Biase (N1), Falconara Albanese (N2) and Cerchiara (N3) (Fig. 1). To minimize the effects of soil and vegetation types on our measurements, we chose sites of matched vegetation types. All sites consist of calcareous, dry grasslands (Festuco-Brometalia); *Spartium junceum* L., *Cytisus sessilifolius* L. and *Cistus incanus*

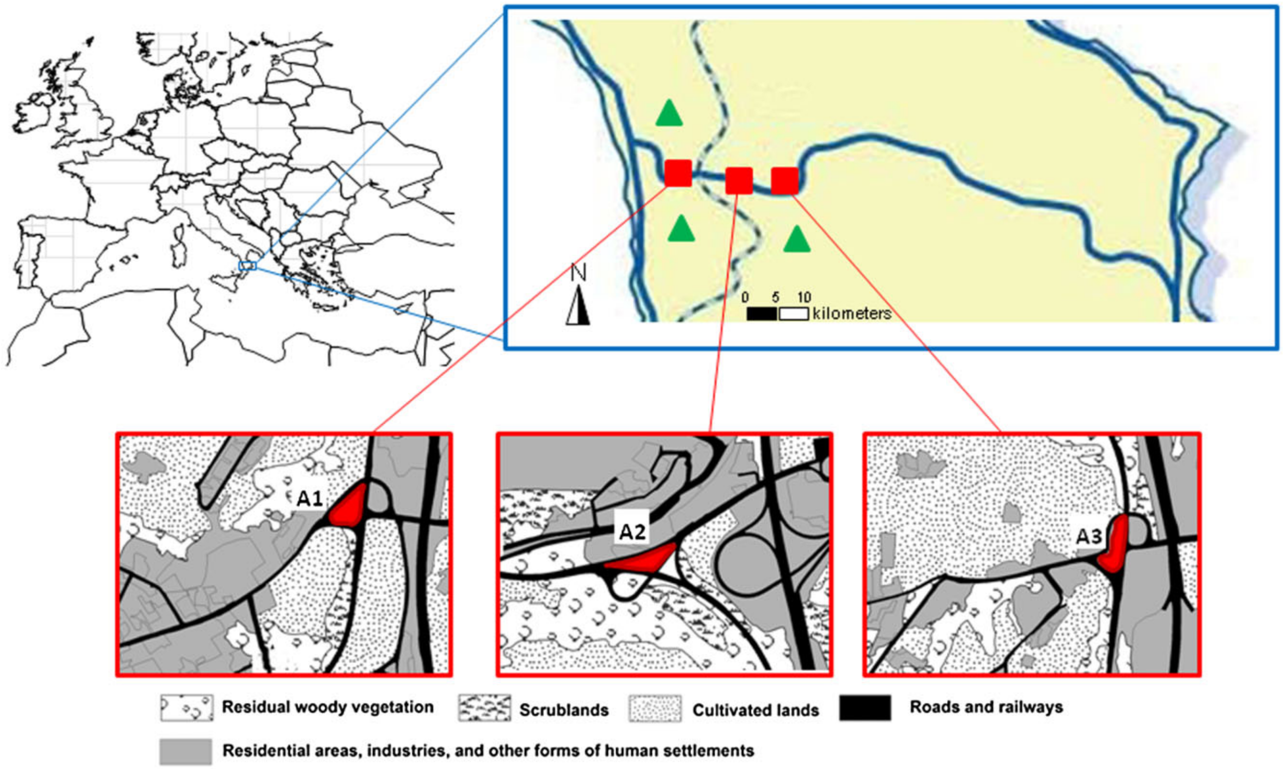

Figure 1. Location of anthropic (square) and natural (triangle) populations used to study the effects of habitat fragmentation on reproductive success and population viability of *Serapias cordigera*.

L. are frequent shrubs and *Festuca circummediterranea* Patzke, *Bromus erectus* Huds. and *Dactylis glomerata* L. are the dominant herbs. The only disturbance between the anthropic and natural populations is the presence of roads close to the anthropic populations. Indeed, during 14 years of observations no populations were mown, or destroyed by fire or by sheep grazing. Moreover, to verify environmental differences independent of human disturbance (e.g. soil composition, temperature or potential evapotranspiration) at each site, climate variables of monthly rainfall and temperature were examined each year in five months (February, April, June, August and October). These months were selected to represent climatic conditions for the whole year. In addition, texture, pH and organic matter of the soil of each population were analyzed. Soil samples were taken from the upper 10 cm of the soil surface, with a 50-mm-diameter soil core sampler. For each population, ten soil core samples were randomly collected. Three core samples were mixed thoroughly to get a field-moist composite sample and were used to analyze soil pH in distilled water (soil/solution ratio 1:2.5). Organic carbon was determined by wet oxidation, and total nitrogen was determined by Kjeldhal digestion (Digestion System 40, Kjeltec Auto 1030 Analyzer). The granulometric composition was determined using the pipette method (Gee & Bauder, 1979). Sand was separated by sieving, clay was quantified by sedimentation based on Stoke's law and silt was calculated by the difference between total soil mass and sum of clay plus sand.

In 1999, a 10 m × 10 m plot in the centre of each population was permanently marked. From 1999 until 2012, in each year and in each population, we individually marked and counted the number of individuals, classifying them by developmental and reproductive status into five stage classes: seedlings (new plants not recorded in the previous year); vegetative rosettes; flowering individuals; reproductive plants (inflorescences with at least one fruit); and dormant/dead. We registered the first appearance, which does not correspond to the year of germination as orchid seedlings remain for a period below-ground after their germination (Arditti & Ghani, 2000; Whigham *et al*., 2006), but in any case for all examined plants, ages and lifespans were calculated from first appearance. In some Mediterranean orchids the root-tuber is replaced each year, gradually increasing in size, and first flowering occurs only after several years, after accumulation of a minimal critical mass (Tatarenko & Kondo, 2003). Orchids can show dormant periods and thus plants recorded in previous years may fail to appear above-ground for one or more consecutive years, after which they re-emerge. Several authors have defined an orchid as ‘dead’ if it failed to emerge for three consecutive years (Hutchings, 1987; Kull, 2002), although longer dormancy is documented in some orchids (Shefferson, 2009). We have no information about the dormant period of *Serapias*, and thus plants that did not reappear were registered as dormant/dead.

In each year in each population, the number of flowers which produced fruits was counted and these data were used to calculate reproductive success in each year by dividing produced fruits by the number of available flowers.

DATA ANALYSIS

Normally distributed values of the five stage classes (seedlings, vegetative, flowering, reproductive and dormant/dead plants) and reproductive success (fruit set) between and within environmental conditions (anthropic vs. natural) were analyzed using a repeated-measures analysis of variance (ANOVA), with environmental conditions, populations and annual census as fixed factors. When the *F*-test was significant, means were compared using Tukey’s test at 5% error probability. The variation patterns of the stage classes in relation to the annual census were analyzed by a general linear model (GLM) that incorporated environmental conditions and sites as fixed factors, and the number of plants and flowers as a covariate.

Soil data were analyzed by ANOVA (with environmental conditions and populations as factors). Levene’s test was used to verify the equality of variances among groups. When homoscedasticity (homogeneity of variance) was detected, significant differences between means were established (at $P < 0.05$) using Bonferroni’s test for multiple comparisons. One-way ANOVA and least significant difference post-hoc tests were conducted to detect differences in monthly rainfall and temperature between sites.

Statistical analyses were carried out using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA).

To assess the future prospects of this species and to evaluate the dynamics of examined populations, a count-based PVA was conducted using Vortex software ver. 9.50 (Miller & Lacy, 2005) and the following equation (Dennis, Munholland & Scott, 1991):

$$N_{t+\tau} = N_t(\mu\tau + \varepsilon),$$

where $\varepsilon \sim N(0, \sigma^2\tau)$.

The parameters μ and σ determine the rate at which the median increases through time and the rate of spread of the distribution, respectively. When censuses are conducted yearly the maximum-likehood estimates of μ and σ are:

$$\mu = \text{mean}[\ln(N_{t+1}/N_t)]$$

and

$$\sigma^2 = \text{var}[\ln(N_{t+1}/N_t)].$$

The most recent population count was used as initial population size (n_q), and two individuals as quasi-extinction thresholds (n_e). The probability (π) and mean time (θ) of extinction can be calculated as:

$$\pi = (n_e/n_q)^{2\mu/\sigma 2} \quad \text{and} \quad \theta = \text{Xd}/|\mu|.$$

We constructed demographic transition matrices for each population (six) × stage classes (four) × interval year (13) using the projection matrix function in the popbio package in R (Stubben & Milligan, 2007). The life cycle of *S. cordigera* can be regarded as consisting of four stages: seedlings (S); vegetative rosettes (V); flowering individuals (F); and dormant individuals (D) (Fig. 2). In addition, we added transition between flowering individuals (F) and reproductive plants (R) (inflorescences with at least one fruit). These matrices contain the probabilities of individuals to transition from stage *x* in year *t*–1 to stage *y* in year *t*.

We calculate stochastic population dynamics following the methods suggested by Tuljapurkar, Horvitz & Pascarella (2003) using the initial vector N(*t*):

$$\text{N}(t) = \text{X}(t)\text{N}(t-1),$$

where X(*t*) represents values of the life-history transition matrix between time *t*–1 and time *t*.

The stochastic growth rates were calculated for each single-year matrix from the following equation from simulations with 100 000 time steps (Tuljapurkar *et al.*, 2003):

$$\log\lambda_S = \lim_{t\to\infty}(1/t)\log[\text{P}(t)/\text{P}(0)]$$

Where the total population size at time t [P(*t*)] is the sum of the elements of vector N(*t*) [N(*t*) = X(t)N(*t*-1)] containing the distribution of individuals in the population between different size categories at a particular time t (Caswell, Neubert & Hunter, 2011). Moreover, we examined significant differences between populations using chi-square tests and the resulting *P* values were subject to sequential Bonferroni adjustment.

RESULTS

POPULATION SIZE AND STRUCTURE

Statistical analysis revealed no significant variation of physical and chemical composition of soil among populations (Table 1). Particle size analysis showed that all soil horizons from the examined populations are dominated by coarse fractions (mainly sand and subordinate silt) with low clay content. Soil analyses

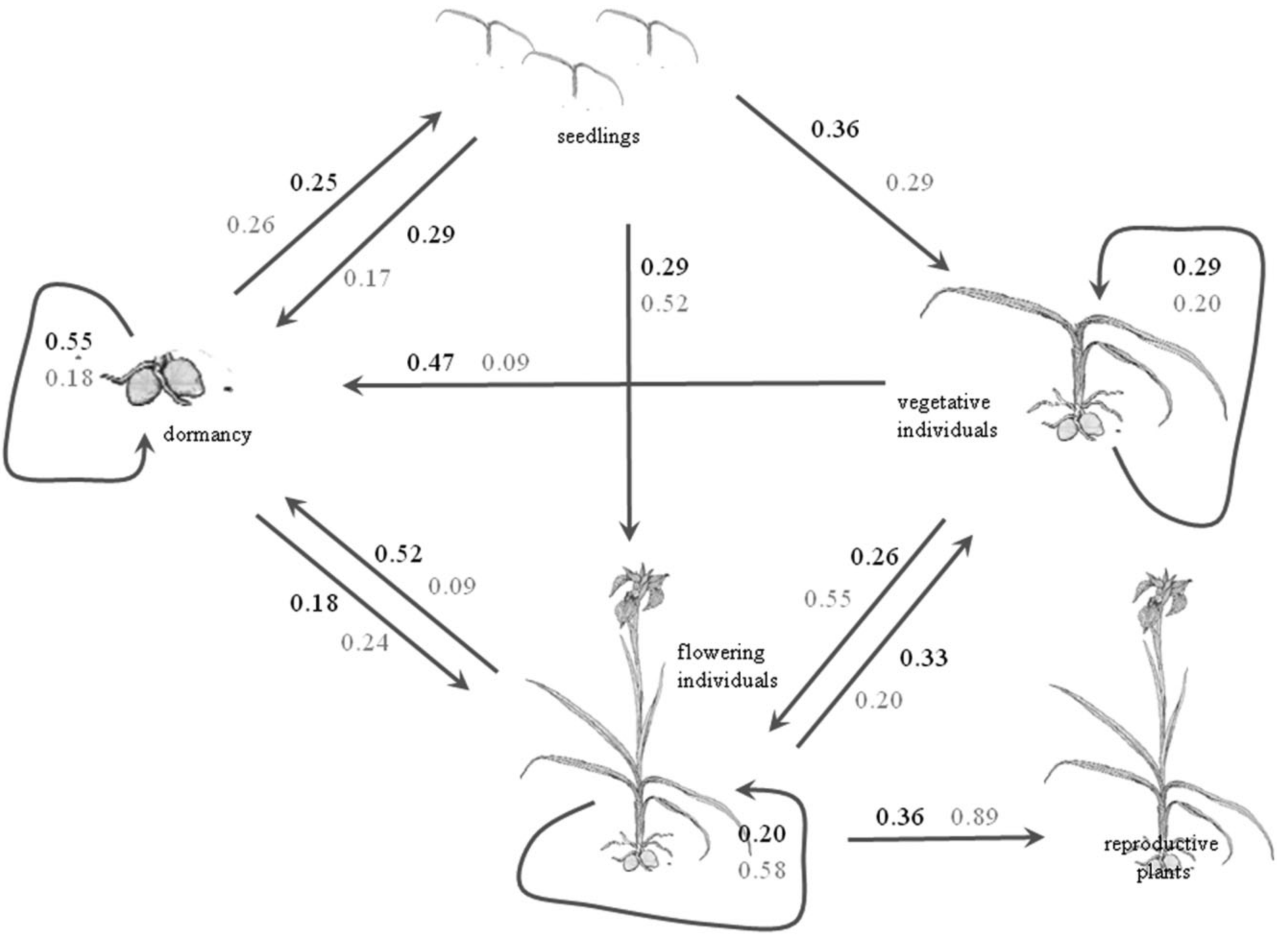

Figure 2. Life cycle graph of *Serapias cordigera*. Stage classes: seedlings (S), vegetative (V), flowering (F), dormant (D) individuals and reproductive plants (R). Arrows show possible transitions between stage classes and numbers show probability of transitions in anthropic (black) and natural (grey) populations.

Table 1. Physical and chemical features of three anthropic (A1, A2 and A3) and three natural (N1, N2 and N3) populations

	Particle size distribution					
Population	Sand (%)	Silt (%)	Clay (%)	pH	Organic carbon (g kg^{-1})	Organic nitrogen (g kg^{-1})
A1	58.25	25.42	16.33	5.95	12.80	1.05
A2	62.12	27.29	10.59	6.05	12.22	0.95
A3	61.85	26.79	11.36	5.90	11.67	1.10
Mean	60.74 ± 1.78	26.50 ± 1.05	12.76 ± 2.12	5.95 ± 0.05	12.23 ± 2.02	1.03 ± 0.33
N1	60.55	27.47	11.98	6.10	12.06	1.05
N2	59.68	27.97	12.35	5.90	13.02	1.15
N3	63.74	26.19	10.07	6.15	12.44	1.05
Mean	61.32 ± 1.89	27.21 ± 1.15	11.46 ± 1.98	6.05 ± 0.05	12.50 ± 2.14	1.08 ± 0.45
ANOVA d.f. (5,70)	0.698$^{\text{ns}}$	0.721$^{\text{ns}}$	0.678$^{\text{ns}}$	0.524$^{\text{ns}}$	0.407$^{\text{ns}}$	0.387$^{\text{ns}}$

d.f., degrees of freedom, ns, not significant.

did not show significant differences in pH and organic matter among the six populations (Table 1). Organic carbon is generally high, with values ranging between 11.67 g kg^{-1} and 13.02 g kg^{-1}, and organic nitrogen showed lower values (0.95–1.15 g kg^{-1}). Soil reaction is generally moderately to slightly acid in all soil horizons, reaching a minimum pH (H_2O) value of 5.9 (Table 1).

Mean annual rainfall and temperature were calculated for the 14 years of the study (1999–2012) according to monthly data for each population (Table S1). ANOVA showed no significant differences in precipitation and temperature among populations, which were characterized by rainy and cold winters, and dry and warm summers.

Based on ANOVA, human activities produced strong effects on the number of total, flowering and dormant/dead plants. The six populations examined showed a significant variation in the number of total and flowering plants within the permanent plots and among years (Table S2). None of the examined stage classes (seedling, vegetative, flowering, reproductive, dormant/dead, total plants and percentage of fruits) differed significantly among the three anthropic or the three natural populations (Table 2). An anthropic population (A1) showed the lowest mean values of the number of total (82.07 ± 4.33) and flowering (78.14 ± 3.78) plants, and a natural population (N1) showed the highest mean values (total plants, 819.86 ± 12.22; flowering plants, 814.57 ± 14.02) (Table 3).

Moreover, in anthropic populations from 1999 onwards, the number of total plants and flowering plants decreased to reach low values in 2012 (Fig. 3A, B). Indeed, the anthropic populations showed a significant, progressive decrease of ~ 3–4% per year in the number of total (Fig. 3A), and flowering (Fig. 3B) plants. The natural populations showed a slight increase in the number of total and flowering plants (Fig. 3A, B), with a mean percentage increase of ~ 2% per year. There was a significantly negative correlation for the anthropic populations (A1 $r_s = -0.82$, $P < 0.001$; A2 $r_s = -0.76$, $P < 0.001$; A3 $r_s = -0.71$, $P < 0.001$) among the number of flowering plants and the year of observation, and a significantly positive correlation for the natural populations (N1 $r_s = 0.79$, $P < 0.001$; N2 $r_s = 0.82$, $P < 0.001$; N3 $r_s = 0.73$, $P < 0.001$). The replicate ANOVAs did not show significant differences (Table 2) in the number of vegetative plants (ranging from 2.5 ± 0.35 to 4.43 ± 0.65) or in the number of seedling plants ranging from (0.14 ± 0.02 to 1.29 ± 0.05).

The proportion of established plants (seedlings and total plants pooled) that survived to the next census in *S. cordigera* populations was higher in the natural populations than in the anthropic populations

Table 2. ANOVA illustrating patterns of variation of seedling, vegetative, flowering, reproductive, dormant/dead, total plants and percentage of fruits among environmental conditions (anthropic vs natural populations), years of observation and populations

	Seedling plants		Vegetative plants		Flowering plants		Reproductive plants		Dormant/dead plants		Total plants		Percentage of fruits	
Source of variation	d.f.	*F*	d.f.	*F*	d.f.	*F*	d.f.	*F*	d.f.	*F*	d.f.	*F*	d.f.	*F*
Environmental	1, 82	1.004^{ns}	1, 82	0.798^{ns}	1, 82	7.214**	1, 82	3.711**	1, 82	3.454**	1, 82	7.362**	1, 82	4.333**
Years	13, 70	0.764^{ns}	13, 70	1.023^{ns}	13, 70	6.580**	13, 70	4.812**	13, 70	4.343**	13, 70	6.545**	13, 70	5.324**
Populations	5, 78	0.905^{ns}	5, 78	0.674^{ns}	5, 78	1.021^{ns}	5, 78	0.874^{ns}	5, 78	0.435^{ns}	5, 78	0.632^{ns}	5, 78	0.847^{ns}

*Significant at 0.05.
**Significant at 0.01.
d.f., degrees of freedom; ns, not significant.

Table 3. Annual census values in three anthropic (A1, A2 and A3) and three natural (N1, N2 and N3) populations

Populations	No. of seedling plants	No. of vegetative plants	No. of flowering plants	No. of reproductive plants	No. of dormant/ dead plants	No. of total plants	Percentage of reproductive plants	No. of flowers	No. of fruits	Percentage of fruits
A1	0.14	3.79	78.14	25.07	25.43	82.07	30.42	574.93	72.71	11.80
	(0.03)	(1.03)	(3.78)	(4.02)	(3.98)	(4.33)	(2.45)	(12.44)	(14.76)	(2.41)
A2	0.21	3.57	106.43	45.36	24.29	110.21	42.29	783.71	82.36	10.22
	(0.07)	(1.13)	(3.45)	(3.42)	(4.02)	(6.33)	(2.78)	(13.45)	(12.88)	(2.09)
A3	0.14	3.00	116.71	37.57	23.43	119.86	31.79	859.43	85.14	9.67
	(0.02)	(1.07)	(4.21)	(3.432)	(3.42)	(6.53)	(2.55)	(13.05)	(14.15)	1.22
Mean	0.16	3.45	100.42	36.00	24.38	104.05	34.83	739.35	80.07	10.56
	(0.05)	(1.02)	(3.20)	(4.04)	(3.83)	(6.82)	(2.75)	(13.95)	(14.45)	(2.02)
N1	0.86	4.43	814.57	728.86	0.43	819.86	89.47	6028.64	4570.07	76.06
	(0.13)	(0.65)	(14.02)	(14.02)	(0.22)	(12.22)	(4.45)	(42.08)	(23.95)	(4.45)
N2	0.71	2.50	590.64	530.79	0.21	593.87	89.97	4348.93	3355.29	77.41
	(0.09)	(0.35)	(13.82)	(12.42)	(0.13)	(10.88)	(4.13)	(40.45)	(22.47)	3.42
N3	1.29	2.50	782.43	705.29	0.29	786.21	90.19	5761.43	4388.36	76.44
	(0.05)	(0.35)	(14.22)	(13.76)	(0.14)	(12.44)	(4.76)	(39.65)	(25.45)	(3.98)
Mean	0.95	3.14	729.21	654.98	0.31	733.31	89.88	5389.67	4104.57	76.64
	(0.09)	(0.38)	(14.52)	(14.31)	(0.16)	(13.02)	(4.47)	(42.45)	(25.33)	(4.35)

Values are given as mean (standard error).

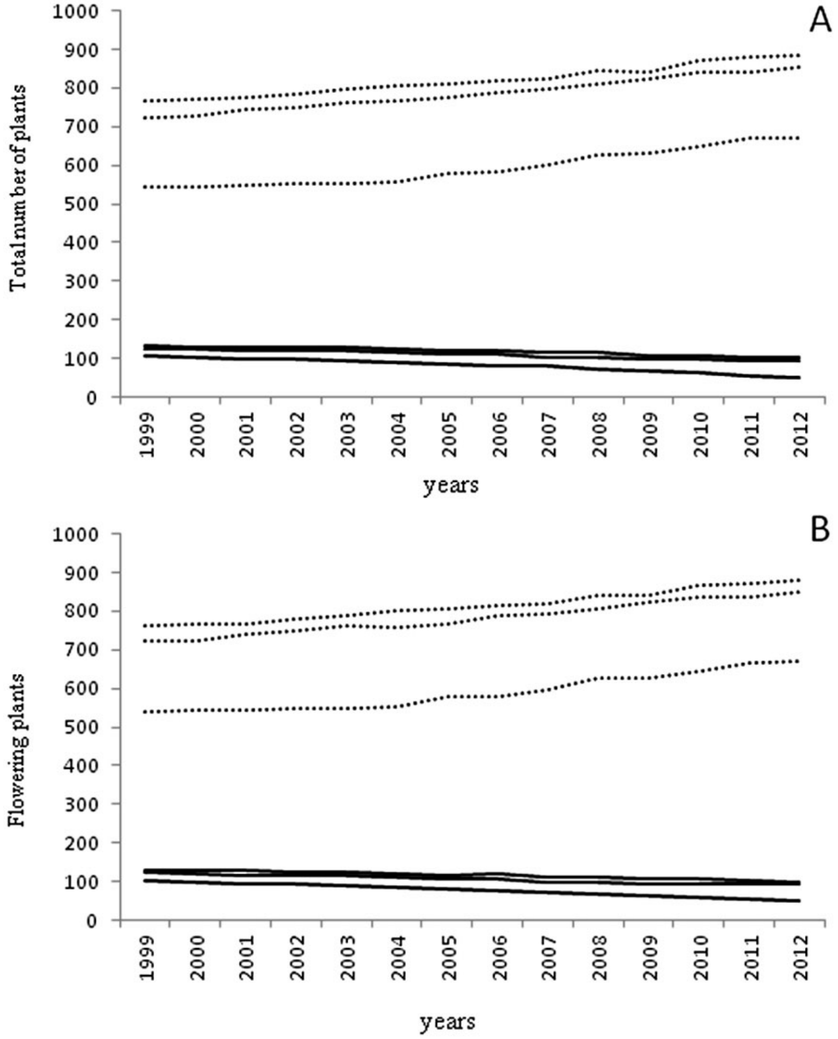

Figure 3. The number of total (A) and flowering (B) plants recorded in three natural (dotted lines) and three anthropic (continuous lines) populations of *Serapias cordigera* each year from 1999 to 2012.

(chi-square test, $P < 0.05$ after Bonferroni adjustment). In natural populations the transition probabilities from seedling, vegetative and flowering individuals to flowering individual (S–F, V–F and F–F, respectively) were higher than in anthropic populations, and anthropic populations showed higher probability of transition from dormant, vegetative and flowering individuals to dormancy (D–D, V–D and F–D, respectively) (Fig. 2). In natural populations the stochastic growth rates (λ_S) were higher (from 1.416 to 0.896, mean value 1.186 ± 0.24) than those of anthropic populations. In anthropic populations λ_S were below the value of 1, indicating that population sizes decline more quickly in disturbed than in natural conditions (Table S3).

In the PVA, starting population sizes for the anthropic populations of 51, 94 and 102 (the population size of the last population census of the three anthropic populations) and a quasi-extinction threshold of two individuals were used; this resulted in ultimate extinction probabilities of 0.656, 0.804 and 0.887, respectively, giving times to extinction of 15, 25 and 40 years, respectively.

REPRODUCTIVE PLANTS AND FITNESS

ANOVA showed that the mean number of reproductive plants varied significantly among populations and among years with no significant variation within sites (Table 2). The anthropic populations A1 and A3 showed lower values (30.42 ± 2.45% and 31.79 ± 2.55%, respectively) than did natural populations (mean value: 89.88 ± 3.20%). Moreover, there was a significantly negative correlation for anthropic

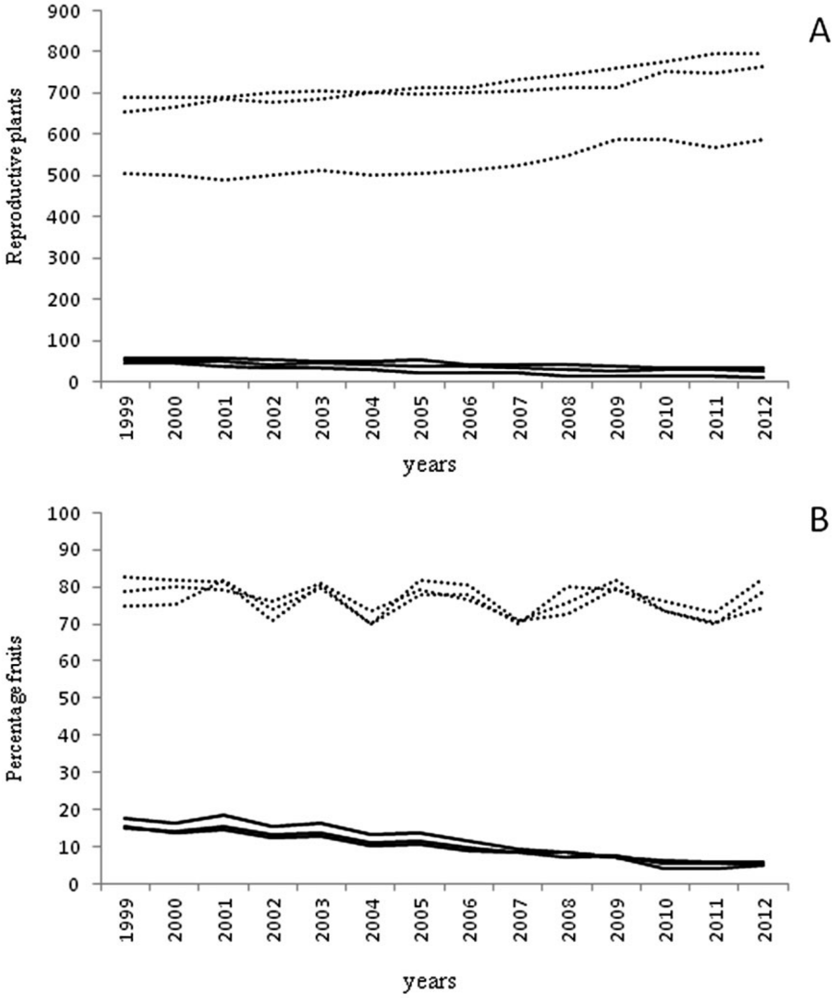

Figure 4. The number of reproductive plants (A) and fruit percentage (B) of *Serapias cordigera* between 1999 and 2012 at three natural (dotted lines) and three anthropic (continuous lines) populations.

populations (A1 $r_s = -0.32$, $P < 0.001$; A2 $r_s = -0.36$, $P < 0.001$; A3 $r_s = -0.31$, $P < 0.001$) among the number of reproductive plants and year of observation (Fig. 4A), whereas there was a significantly positive correlation for natural populations (N1 $r_s = 0.59$, $P < 0.001$; N2 $r_s = 0.62$, $P < 0.001$; N3 $r_s = 0.63$, $P < 0.001$) (Fig. 4A). Indeed, in anthropic populations, the number of reproductive plants decreased from 45–58 in 1999 to 10–32 in 2012 (Fig. 4A).

The percentage of fruits produced within populations did not show significant variation (Table 2), but varied significantly among environmental conditions and years (Table 2), with a mean value ranging from 9.67 ± 1.22 in population A3 to 77.41 ± 3.42 in population N2 (Table 3). Anthropic populations showed a significant and negative correlation among values of fruit set and year of observation (A1 $r_s = -0.92$, $P < 0.001$; A2 $r_s = -0.86$, $P < 0.001$; A3 $r_s = -0.82$, $P < 0.001$), and thus there was a strong decline in the percentage of fruit production, with a decrease from 18.45% to 3.92% (Fig. 4B). On the contrary, natural populations showed constant values of percentage of fruit among years of observation and no correlation between fruit set and years of observation (Fig. 4B).

Performing a PVA using the last number of reproductive plants (10, 32, 27) as the population size, the extinction probabilities were 0.522, 0.602 and 0.674, respectively, giving times to extinction of 9, 18 and 22 years, respectively.

DISCUSSION

Our results provide detailed information on the negative effect of human activities on temporal fluctuations, reproductive success and population dynamics in a group of populations of *S. cordigera*. Anthropic populations showed significantly lower values of reproductive success and the proportion of established plants that survived to the next census stage classes than did natural populations. Possible explanations for such a decline over the last 14 years are habitat fragmentation by human activities and changes in pollinator activity. *Serapias cordigera* is a Euro-Mediterranean orchid that has declined in population size and area of distribution at regional and local scales throughout its range (in particular, in much of southern Europe), probably as a result of habitat loss and habitat reduction (Pellegrino *et al*., 2007; Feldmann & Prat, 2011). Our 14 years of accurate estimations of population size and year-to-year observations showed considerable variation in the proportion of plants flowering and reproductive success in the orchid populations examined. The size of the natural populations of this study ranged from 542 to 854 plants, and ~ 85–90% of them produced flowers. These annual censuses in the natural plots are similar to those in other Mediterranean populations of *Serapias* spp. (Pellegrino *et al*., 2006; Bellusci *et al*., 2010) and indicate stable population trends over the previous years.

The anthropic populations had a lower population size and a lower fruit set than did natural populations, suggesting that the latter populations are better buffered. This is probably a consequence of inadequate pollinator visitation in small populations, resulting in strong pollinator limitation (Smithson, 2005; Tremblay *et al*., 2005). Larger populations of plants are likely to be more attractive to pollinators, resulting in higher visitation rates and therefore pollination success (Mustajärvi, Siikamäki & Rytkönen, 2001), whereas small populations may suffer from insufficient pollen transfer and consequently lower seed set (Xia *et al*., 2012). *Serapias cordigera* did not self-pollinate, and fruit set in hand-pollinated flowers was significantly higher than in open-pollinated flowers (Pellegrino *et al*., 2005), suggesting that differences in reproductive success among populations in this study were probably a result of differential visitation frequency or efficiency of pollinators in urban areas, rather than differences in resource limitation among populations, thus providing a link between pollinator activity and fruit production. There are no differences in organic matter (Table 1) or climatic (rainfall and temperature) conditions between anthropic and natural populations.

No extreme weather events, fire or grazing seem to have disturbed the populations, and thus their decrease in population size and reproductive success probably reflects the effect of human activities (construction of roads) that can alter movements and behaviour of insects (Wilcock & Neiland, 2002; Biesmeijer *et al*., 2006; Potts *et al*., 2006; Vereecken, Cozzolino & Schiestl, 2010). Although insects have the ability to cross a barrier, human structures (e.g. roads and railroads) restrict insect movement (Bhattacharya, Primack & Gerwein, 2003) and therefore interfere with pollinator activity, thus decreasing population size and depressing reproductive success (Tremblay *et al*., 2005; Huang *et al*., 2009). This is especially so for specialist orchid species, which rely on a few pollinators, making them more vulnerable to pollinator limitation (Swartz & Dixon, 2009). The lower fruit production in anthropic populations is probably a result of their lower insect-visitation rates because small and isolated populations may not be found by pollinators (Steffan-Dewenter & Tscharntke, 1999).

Our results support widespread ideas and demonstrate that habitat fragmentation by human activity alters the lifespan of orchids more quickly than do ecological processes and can cause extinction of populations (Coates & Dixon, 2007; Bidartondo & Read, 2008; Swartz & Dixon, 2009; Parra-Tabla *et al*., 2011). Increasing urbanization has resulted in a dramatic reduction in the number and size of natural habitats for orchid species, many of which often remain confined to the edge of roads (Jacquemyn *et al*., 2005; Kull & Hutchings, 2006).

Habitat fragmentation is the most significant factor threatening to cause species extinction in the future (Gurevitch & Padilla, 2004; Fischer & Lindenmayer, 2007). The values of PVA obtained and the demographic data of our anthropic populations clearly showed that the populations near to human constructions are subject to a high risk of extinction. Indeed, the anthropic population with the lowest number of individuals (A1) had a projection of future life that did not exceed 15 years. From a demographic point of view, the populations studied had a chance of surviving for 15–40 years; these prospects may be too optimistic, as it was clearly shown that the population has declined seriously during the last 14 years of monitoring.

Considering that in 1999 the population sizes of the three anthropic populations were 106, 126 and 134 individuals, respectively, and that there was a mean decrease in the population size of ~ 3–4% per year, we can argue that before the start of road construction (c. 1960) the size of these populations could have been 400, 480 and 520 individuals, respectively. This suggests that when the anthropic populations were still natural populations their size was comparable to that of existing natural populations.

Our study provides evidence that human activity has a pervasive effect on the flowering patterns and population dynamics of natural *S. cordigera* populations. The relative stability and persistence of the three natural populations, compared with an alarming decrease both in population size and in fruit set of the three anthropic populations, strongly indicates a high risk of extinction in many populations of *S. cordigera*. These findings should be considered during conservation management planning or during road/railway building planning because habitat fragmentation will clearly have an impact on the survival of plants.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Rainfall and temperature monthly data for the 14 years (1999–2012) for population A1.
Table S1 Rainfall and temperature monthly data for the 14 years (1999–2012) for population A2.
Table S1 Rainfall and temperature monthly data for the 14 years (1999–2012) for population A3.
Table S1 Rainfall and temperature monthly data for the 14 years (1999–2012) for population N1.
Table S1 Rainfall and temperature monthly data for the 14 years (1999–2012) for population N2.
Table S1 Monthly rainfall and temperature data for the 14-year study period (1999–2012) for populations A1–A3 and N1–N3.
Table S2 Values of annual census in three anthropized (A1, A2 and A3) and three natural populations (N1, N2 and N3).
Table S3 Stochastic growth rates (λ_S) and life-history transition matrices in three anthropized (A1, A2 and A3) and three natural populations (N1, N2 and N3) of *S. cordigera*. In the matrix models, we considered four stage-classes: seedlings (S), vegetative rosettes (V), flowering (F), and dormant (D) individuals.