

# Short-term fitness and long-term population trends in the orchid *Anacamptis morio*

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**Abstract** The conservation of endangered species critically depends on the understanding to which degree short-term fitness and long-term trends are affected by intrinsic local conditions and external global dynamics. However, studies combining long-term demographic data with population level analyses of site conditions, genetic variation, and reproduction as well as with climatic data are still rare. Here we studied the endangered orchid *Anacamptis morio*, representative for species with a sub-mediterranean distribution. For populations at the northern range edge, we combined long-term monitoring data (1977–2010) with climatic data and analyzed reproductive fitness components, genetic variation, and abiotic site conditions. Reproduction was generally low as expected from the deceptive pollination system, and was positively influenced by population size and xerothermic site quality. The majority of populations showed a positive population trend, which was paralleled by an increase in spring temperature and positively affected by site quality. High levels of genetic variation were found in the populations which were at gene flow-drift

equilibrium. *A. morio* may profit from increasing spring temperatures because of increased reproductive output. Nevertheless, whether climate change results in fitness increase or not may depend on the maintenance and provision of optimal site quality, i.e., xerothermic and nutrient poor conditions.

**Keywords** Reproduction · Fitness · Genetic variation · Site quality · Climate change · Weather

## Introduction

The conservation of endangered plant species and declining populations critically depends on the understanding of fitness determinants (Oostermeijer et al. 2003). However, fitness measures at population and individual level may vary among years depending on actual biotic and abiotic environmental conditions (Morris et al. 2008), cumulative fitness across larger time scales eventually determines the long-term population trend. Hence, the consequences of site and population characteristics, such as abiotic conditions, population size (Lienert et al. 2002) as well as long-term dynamics such as climatic changes (Feehan et al. 2009) need to be taken into account to assess the sustainability of a population (Magnuson 1990; Oostermeijer et al. 2003). Moreover, the relative contribution of different life-history processes and fitness determinants to long-term performance varies across species (Silvertown et al. 1993; Jongejans et al. 2010)

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and needs to be quantified explicitly to reveal species-specific patterns. Even if these different aspects are not easy to disentangle due to correlation or multicausality in observational studies on endangered species, the potential determinants of fitness have to be addressed to arrive at testable hypotheses.

Abiotic site conditions, such as the soil water relations or nutrients affect plant growth directly or may determine the strength of competitors (Chesson and Huntly 1989). Thus different habitat types, in particular soil type and water availability can strongly affect reproductive performance, individual fitness, and population viability (e.g., Csergö et al. 2011). Also, the spatial relations within and among populations have been shown to be related to fitness (Lienert et al. 2002). Habitat area is likely a proxy for habitat suitability, as it may first be positively correlated to heterogeneity of environmental conditions which may represent a buffer during habitat changes; second habitat area may be inversely related to edge effects, like nutrient input and disturbance (Murcia 1995). Spatial isolation among populations has been shown to be related to plant fitness (Bizoux et al. 2008) as it affects functional connectivity which may be a proxy for similarity of environmental conditions. Finally, landscape composition may affect individual fitness due to habitat connectivity or distance to pollinators (Steffan-Dewenter et al. 2001).

Population size has been shown to be a good predictor of plant fitness and population viability (Leimu et al. 2006). First, population size may be itself related to habitat size and its associated buffering properties mentioned above. Second, population size strongly affects pollination and thus reproductive fitness (Knight et al. 2005). Furthermore, genetic variation often increases with population size which can affect fitness (Ellstrand and Elam 1993; Leimu et al. 2006). For example, reduced genetic variation entails a higher extinction risk when inbreeding has detrimental effects on fitness (Young et al. 1996). Additionally, genetic variation may reflect the inherent evolutionary potential when environmental conditions change and thus may affect long-term population development (Frankham 2005). However, as genetic variation often increases with population size, their independent effects on fitness are difficult to disentangle.

Independent of specific local conditions, weather and climate are additional factors acting on reproduction and survival and, hence, on short- and long-term

population dynamics (Hedhly et al. 2009). Weather conditions during particular time periods, like late frosts or drought may inhibit flowering or fruiting. Moreover, weather conditions can influence the individual resource status or the interactions with pollinators and thus affect reproduction (Jacquemyn et al. 2009). Consequently, varying weather conditions may result in annual fluctuations of reproductive success of individuals or census size of populations (Bernhardt and Edens-Meier 2010). Climate change, however, operates at larger temporal scales upon plant populations and may affect both phenology and individual fitness components, thus leading to altered long-term population dynamics (Sparks and Menzel 2002; Hedhly et al. 2009).

*Anacamptis morio* (Orchidaceae), representative for species distributions centered in the Mediterranean, has experienced drastic population extinctions within the last century in Central Europe (Jersakova et al. 2002; Jacquemyn et al. 2005; Kull and Hutchings 2006). In Central Europe, it is now restricted to isolated grassland habitats and serves as a flagship species for conservation (Böhnert et al. 1986). Like many other orchids, *A. morio* is known to be pollen limited which results in low fruit set (Jersakova and Kindlmann 1998) and may profit from a large display of conspecific or heterospecific flowers (Johnson et al. 2003; Knight et al. 2005). Furthermore, fitness decline in offspring resulting from selfing, i.e., inbreeding depression, suggests an important role of genetic variation for reproduction (Smithson 2006). Although a number of studies focused on pollination ecology, management requirements and demography in *A. morio*, analyses of reproductive fitness in multiple populations are lacking. Such analyses would provide important indications for the formulation of conservation actions. Additionally, the impact of genetic variation, population size, and local site conditions on reproduction and their importance for long-term population dynamics has not been studied in *A. morio*.

In this study, we combine the assessment of reproductive output, genetic variation, and site conditions of remnant populations of *A. morio* with an analysis of long-term monitoring data. We want to elucidate (a) which abiotic, demographic, and genetic population characteristics affect reproductive fitness components in a single year and (b) whether and how census sizes across multiple years are influenced by weather and climatic conditions. We furthermore

assess (c) the long-term trend in population size and its determinants.

## Materials and methods

### Study species and study sites

*Anacamptis morio* (L.) R. M. Bateman, Pridgeon and Chase (syn. *Orchis morio* L.), the Green-winged Orchid, is a small, perennial, wintergreen geophyte. *A. morio* is food deceptive and is mainly visited by early emerging bumblebee queens (Nilsson 1984). The species is self-compatible but relies on pollinator visits to set fruits (Jersakova and Kindlmann 1998). Plants produce one inflorescence, with 15–20(–25) flowers (Fay and Rankou 2010). Fruits contain thousands of wind-dispersed dust-like seeds of which only a small proportion is dispersed more than one meter (Jersakova and Malinova 2007).

*Anacamptis morio* has a wide European distribution centered in the Mediterranean. Across its distribution range, it has a broad ecological range and occurs in various types of grasslands and prefers neutral or calcareous soils (Fay and Rankou 2010). We studied *A. morio* in central Germany where the species has experienced drastic declines in recent decades and is critically endangered (MLRU 1996, Böhnert et al. 1986). In this area, populations are exclusively found in xerothermic grasslands mostly located on isolated porphyry outcrops within the agricultural landscape and most of them undergo conservation management by occasional grazing. The climate of the study area is characterized by low annual rainfall of approximately 460 mm and mean annual temperature of 9.6 °C. Compared to the long-term mean, values slightly higher than average values were observed for precipitation (633 mm) and temperature (9.9 °C) in the study year 2009.

For a total of 31 populations in the study area, long-term monitoring data of the number of flowering *A. morio* plants during peak flowering is available from 1977 onwards, by courtesy of the voluntary working-group for native orchids (Arbeitskreis Heimische Orchideen, AHO Sachsen-Anhalt and AHO Thüringen). The mean number of censuses per population was 21 years (range 9–33 years), as not all

populations were censused each year. Henceforward, this monitoring data is called the long-term dataset.

In 2009, we studied a subset of 19 of these populations for site conditions, genetic variation, and reproductive fitness (Table 1; Fig. 1). We obtained three descriptors of spatial and environmental site conditions for each site. First, we estimated patch area as proxy for edge effects and habitat heterogeneity. Based on a topographical map (1:25000, Landesvermessungsamt Sachsen-Anhalt) and additional digitization, habitat area (0.15–131 ha, Tab. 1) was assessed with Arc GIS 10 (ESRI 2011, ArcGIS Desktop: Release 10, Redlands, CA: Environmental Systems Research Institute). Second, we calculated Hanski (1994) connectivity index:  $CI_i = \sum_{i \neq j} \exp(-\alpha d_{ij}) A_j^b$ , where  $A_j$  is the census size 2009 of population  $j$  and  $d_{ij}$  is the edge to edge distance (km) between populations  $i$  and  $j$ . We chose  $\alpha = 2$  for the effect of distance to migration ( $1/\alpha$  is the average migration distance) and  $b = 0.5$  for the scaling parameter (Moilanen and Nieminen 2002). Third, we computed a site quality index summarizing local environmental conditions based on occurrence data of vascular plant species at the respective sites (D. Frank, pers. comm., Hornemann unpubl. data) and the species' mean Ellenberg indicator values for light, soil moisture, temperature, soil mineral nitrogen, soil reaction (pH), and continentality (Ellenberg et al. 1992). In general, this approach has proven suitable to indicate the prevailing environmental site conditions (Dupré and Diekmann 1998; Schaffers and Sýkora 2000). We performed a principal component analysis (PCA) of the mean indicator values. The first PCA axis accounted for 65 % of the variation and its scores were used as site quality index (Table 1). The site quality index was negatively correlated to nitrogen and moisture, and positively correlated to light, temperature, continentality, and soil reaction.

In each population, we assessed the total number of flowering individuals in 2009 (census size 2009 hereafter). The number of flowering individuals in a population varies across years and is affected first in the short term by changes in the proportion of adult individuals that actually come into flower and second in the long term by demographic changes in the number of adult individuals. Thus, the census size per year is not an unbiased estimator of population size. For *A. morio*, both management practices and weather

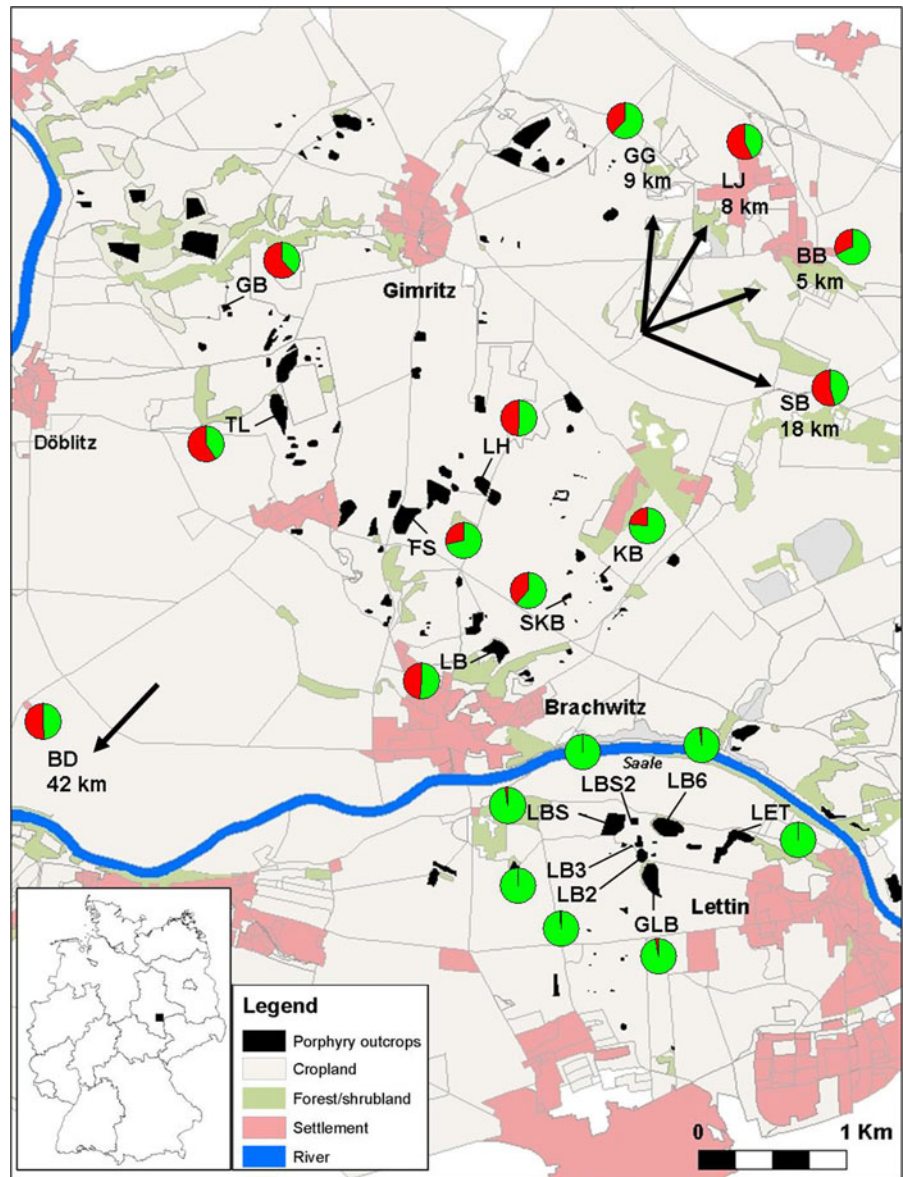
**Table 1** Sampling sites, census size, genetic variation, and reproductive fitness of 19 remnant populations of *Anacamptis morio* in central Germany

Site code	Lat/Long	Habitat area (m <sup>2</sup> )	Site quality	Connectivity	Mean census size (1977–2010)	Nr of censuses	Census size 2009	$\mu$	$n$	$H_E$	PLP	$B_R$	Flowers/plant	Shoot survival (%)	Fruit set (%)	Seed weight (mg)	Embryo formation (%)	Cumulative fitness
LBS	51.531/11.887	20,869	-0.681	57.58	1,613	22	8,375	0.095*	30	0.186	49.5	1.39	11.6	73.3	37.2	11.11	80.10	207,014.90
LBS2	51.531/11.888	2,695	-0.243	82.25	10	15	24	0.087*	23	0.158	38.8	1.33	8.8	43.5	26.6	4.22	67.30	21,569.16
LB3	51.530/11.889	1,472	1.235	116.43	208	21	1,292	0.077	30	0.168	47.6	1.38	9.8	86.7	39.7	8.94	76.10	203,705.80
LB6	51.530/11.892	20,699	-0.851	53.57	17	16	103	0.128*	29	0.193	49.5	1.40	9.7	60.0	50.6	6.54	73.60	91,984.75
LB2	51.529/11.890	3,336	-0.487	154.87	18	12	58	0.111	28	0.169	42.7	1.35	8.1	60.0	36.4	5.21	78.50	65,351.86
GLB	51.528/11.891	18,312	0.847	67.35	136	20	856	0.113	28	0.186	54.4	1.35	10.7	80.0	30.5	5.54	81.60	97,922.55
LET	51.529/11.898	33,165	0.825	24.95	11	9	19	-0.029	16	0.135	34.0	1.30	8.0	68.8	46.7	5.53	77.20	92,918.91
LJ	51.634/11.924	13,900	-2.160	3.82	6	21	8	0.022	7	0.220	57.3	1.57	8.8	37.5	62.0	2.48	79.60	40,253.17
BB	51.586/11.940	30,688	1.929	4.70	3,297	27	5,012	0.092*	29	0.269	71.8	1.61	9.4	53.3	27.8	6.30	80.00	43,071.83
GB	51.562/11.849	3,055	0.984	9.52	771	26	2,394	0.159*	27	0.257	74.8	1.68	10.6	83.3	40.3	5.87	78.10	141,348.30
TL	51.555/11.854	21,636	-2.439	11.79	69	27	77	0.034	29	0.255	72.8	1.66	8.0	40.0	41.1	5.82	68.90	24,367.23
LH	51.551/11.874	6,505	-0.723	15.21	36	31	73	0.046*	28	0.282	76.7	1.67	6.7	66.7	48.0	6.05	70.90	65,276.98
BD	51.308/11.414	1,316,000	4.329	1.00	12,901	14	20,388	0.131	28	0.260	73.8	1.65	9.3	80.0	31.1	6.15	73.70	86,920.29
FS	51.549/11.866	20,485	0.451	15.01	32	25	49	0.041	27	0.253	61.2	1.53	7.5	51.6	52.9	4.29	67.10	47,223.03
LB	51.542/11.875	27,810	1.448	16.40	2,650	31	3,773	0.055*	27	0.263	69.9	1.61	10.2	73.3	40.8	7.08	69.00	136,629.60
KB	51.545/11.886	2,464	0.128	18.75	59	28	17	-0.028	13	0.268	59.2	1.58	8.2	84.6	66.0	4.19	53.90	94,204.24
SKB	51.545/11.882	2,027	-0.819	19.54	47	27	76	0.076	29	0.287	71.8	1.64	8.0	63.3	37.8	4.56	68.20	43,741.97
GC <sup>a</sup>	51.643/11.888	2,394	1.021	3.44	27	25	30	-0.024	28	0.290	76.7	1.65	7.6					
SB	51.520/12.125	12,500	-4.795	2.20	35	32	19	-0.022	16	0.259	64.1	1.63	10.2	42.1	23.2	4.47	63.30	20,383.44
Mean								0.061		0.229	60.3	1.52	9.0	63.8	41.0	5.80	72.62	84,660.45

Site quality = PCA scores of axis 1 of Ellenberg indicator values, connectivity = Hanski's connectivity index, census size = number of flowering individuals,  $\mu$  = population trend (asterisks show significant trends),  $H_E$  = expected heterozygosity, PLP = percentage of polymorphic loci,  $B_R$  = band richness, flowers = nr of flowers per plant, shoot survival = percentage individuals that did not wither until time of fruit ripening, fruit set = fruits/flowers, seed weight = seed weight per fruit (mg), embryo formation = percentage seeds with embryo

<sup>a</sup> No reproductive fitness estimate due to grazing

**Fig. 1** Location of the 19 *A. morio* populations studied. Pie charts denote population level cluster membership coefficients of the Bayesian cluster analysis. For five populations that are located outside of the main study region, the direction from (black arrows) and distance (in km) to the main region is given



conditions have been shown to affect the proportion of individuals that produce flowers (Silvertown et al. 1994; Wells et al. 1998; Jersakova et al. 2002). Thus, year-to-year variation in census size likely reflects the impact of temporally varying weather conditions rather than changes in population size. However, assuming that variation in the proportion of flowering individuals occurs randomly across years, census size data across multiple years can be used to assess temporal population trend.

### Genetic variation and population structure

In each population, we arbitrarily selected and marked 30 flowering individuals, if available, and took a leaf sample for genetic analysis. We studied genetic variation with amplified fragment length polymorphisms (AFLP). DNA was extracted using the DNeasy 96 plant kit (Qiagen, Hilden, Germany). AFLP analyses followed the AFLP plant mapping protocol (Applied Biosystems, Foster City, CA) with minor

modifications. After screening of 60 primer combinations, four were selected and used for selective amplification with fluorescent labeled primers (ACT/CAC, ACA/CTG, AAG/CTGT, AGG/CTG). Fragment analysis was performed on an ABI 3130 genetic analyzer (Applied Biosystems) with GeneScan 600 LIZ as internal size standard. Scoring of fragments was done manually in GeneMapper (version 3.7) and revealed a total of 103 polymorphic loci (PLP) in 515 individuals. Based on 22 replicate samples the error rate was 3.7 %.

Overall genetic population structure was assessed by Bayesian clustering to test for the existence of spatio-genetic groups (see supplemental information for details). In short, we used STRUCTURE v. 2.3 to determine the most probable number of genetic groups by taking into account sampling location (Hubisz et al. 2009). As two spatially structured genetic groups were found (see below), we used, for each population, the proportion of membership to the first group,  $Q$ , as a measure of spatio-genetic affiliation in the later analysis of fitness components. For each population, we assessed genetic variation as percentage of PLP and expected heterozygosity ( $H_E$ ) following (Lynch and Milligan 1994) with AFLP-SURV 1.0 (Vekemans 2002) applying the square root method and assuming Hardy–Weinberg equilibrium. Additionally, we calculated band richness ( $B_R$ ), which is the mean number of phenotypes expected at each locus standardized to the smallest sample size ( $n = 7$ ) by means of a rarefaction method, using *aflp-div* 1.0 (Coart et al. 2005). We tested for correlation of genetic variation with mean census size (1977–2010). Population differentiation was determined as overall and pairwise  $F_{ST}$  in AFLP-SURV. In order to test for isolation-by-distance, we regressed pairwise genetic differentiation  $F_{ST}/(1-F_{ST})$  on log geographic distances. Significance was tested by a Mantel test.

#### Short-term fitness components

In each of the 19 populations, we measured reproductive fitness components in 2009. For all marked individuals we counted (1) the number of flowers per plant. At the time of fruit ripening, we recorded (2) shoot survival, as a part of the shoots withered and (3) the number of fruits per plant. For surviving shoots, fruit set was calculated as percentage of flowers that turned into fruits. We arbitrarily collected one ripe

fruit per plant and determined (4) total seed weight. As a measure of pollination success, we estimated (5) the percentage of seeds containing an embryo. Therefore, the percentage of fully developed seeds was determined by visually analyzing 120 seeds per fruit using a binocular. As an integrative measure of fitness, we calculated (6) cumulative fitness as the product of the means of shoot survival, number of flowers, fruit set, seed weight, and embryo formation.

We performed standardized multiple regression analyses to explain fitness components and cumulative fitness of populations as a function of demographic, spatial, environmental, and genetic predictor variables. Prior to multiple regression analysis, we performed a PCA of the descriptors census size (2009), habitat area, connectivity (CI), site quality index, PLP,  $H_E$ ,  $B_R$ , and  $Q$ , and discarded highly collinear variables. Minimum adequate models were selected using AIC and the *step*-function in R.

#### Weather effects on census size

Using the long-term dataset, we assessed the impact of weather conditions on census size across years in 31 populations. From data of the local weather station Halle-Kröllwitz (German Weather Service), we extracted the mean monthly precipitation and temperature during four time periods in a year which we hypothesized to affect census size: September–October of the previous year (development of winter leaves), December–February (persistence of winter leaves), April (vegetative growth, bulb regeneration), and May (flowering, fruit production). The log census size was then explained by these weather variables using a linear mixed-effect model (*lme*) for repeated measurements. In order to account for temporal pseudoreplication, the sampling unit (Year) was included as random effect. We included the eight weather variables and *Year* as main effects into the model and successively removed non-significant terms to obtain a minimal adequate model.

#### Population trend

Based on the long-term dataset we computed the long-term trend in population size ( $\mu$ ) for 31 populations which had been censused in 9–33 years (mean 21) in the period between 1977 and 2010.  $\mu$ , defined as the average change in log-abundance per unit of time, was

estimated as the slope of a log-linear regression of log census size against year as implemented by Humbert et al. (2009). We used a statistical model that takes into account both, observational error, and environmental stochasticity (EGSS-REML, Humbert et al. 2009). The significance of the population trend is given by the significance of the linear model.

For the subset of 19 populations studied in detail, we performed a multiple regression analysis as described above to explain the population trend  $\mu$  by taking into account habitat area, site quality index, and CI, assuming these explanatory variables to be constant across years. Census size and other temporally variable predictors were not included as independent variable in this model.

If not stated otherwise, all analyses were performed in R, version 2.13.0 (R Development Core Team 2011).

## Results

### Genetic variation and population structure

The Bayesian cluster analysis revealed two gene pools and two groups of populations (Fig. S1). Group I comprised a number of spatially close populations harboring only one gene pool. Group II consisted of spatially isolated populations and appeared admixed with equal contributions of the two gene pools (Fig. 1, Fig. S2). Genetic variation ( $H_E$ ) was higher in group II than in group I (Welch two sample  $t$  test:  $t = -10.06$ , d.f. = 11.93,  $p < 0.001$ ), which may indicate a historical bottleneck in the latter. Overall,  $H_E$  and  $B_R$  were not significantly correlated to mean census size but a weak positive trend between  $PLP$  and mean census size was found ( $p = 0.069$ ). Populations were moderately differentiated with an overall  $F_{ST}$  of 0.161 (SE 0.06,  $p < 0.001$ ). Population differentiation followed an isolation-by-distance model and increased with geographic distance ( $r = 0.458$ , Mantel- $p < 0.01$ ) indicating an equilibrium of genetic drift and gene flow. A Mantel test within the northern group II (excluding population BD) also revealed a positive significant relationship ( $r = 0.465$ , Mantel- $p < 0.05$ ).

### Short-term fitness

The 19 populations differed strongly in census size, ranging from eight to more than 20,000 flowering

individuals in 2009. Population level data on components of reproductive fitness are given in Table 1 and indicate overall low reproductive success and large differences among populations in most fitness components. On average, shoot survival was 64 % and fruit set of surviving shoots was 41 %. Thus, overall, only 26 % of flowers developed into fruits. However, embryo formation was high (73 %) and showed low variation among populations.

In the PCA of demographic, spatial, environmental, and genetic predictor variables, the first axis explained 56 % of variation and was strongly loaded by spatial and spatio-genetic structure, (CI,  $Q$ ) and by genetic variation ( $PLP$ ,  $H_E$ ,  $B_R$ ; Fig. S3). As CI,  $Q$ , and genetic variation were highly collinear ( $R^2 > 0.450$ ) reflecting the spatio-genetic structuring present (see above), only CI was used in the subsequent analyses. Multiple regressions analyzing the combined effect of census size 2009, CI, habitat area, and site quality on components of reproductive fitness are shown in Table 2. Census size was the most important factor positively affecting several fitness components and cumulative fitness. Strong effects were also found for site quality which positively affected shoot survival and had a negative effect on number of flowers and seed weight.

In order to eliminate the effect of the spatio-genetic structuring found, we performed separate analyses for a reduced dataset including only populations of group II. Here, PCA did not show collinearity between CI and genetic diversity and hence, these variables were entered as independent effects in the multiple regression analyses. Nevertheless, census size and site quality still had the most prominent effects on several fitness components (Table S1). Additional significant effects were only found for site quality on shoot survival, CI on embryo formation, and genetic diversity on fruit set.

### Weather effects on census size

Several weather variables affected the annual census size in the long-term dataset (Table 3). The number of flowering individuals was positively related to higher temperatures in April and to higher temperatures and increased precipitation in the autumn of the previous year, and was negatively related to temperatures in May. Weather conditions during winter had no effect. In the analyzed time period (1977–2010), April

**Table 2** Effects of census size, *CI*, and local habitat conditions on components of reproductive fitness and population trend analyzed in 19 *Anacamptis morio* populations

	Flowers	Shoot survival	Fruit set	Seed weight	Embryo formation	Cumulative fitness	Population trend ( $\mu$ )
Census size 2009	0.992***	0.348	-0.432 <sup>†</sup>	0.966***	0.446 <sup>†</sup>	0.778**	- <sup>a</sup>
<i>CI</i>				0.008 <sup>†</sup>	0.007 <sup>†</sup>		0.009
Habitat area		-0.397 <sup>†</sup>				-0.406 <sup>†</sup>	
Site quality	-0.563*	0.567*		-0.209 <sup>†</sup>			0.285*
$R^2$ (final model)	0.580***	0.565**	0.187 <sup>†</sup>	0.669**	0.281 <sup>†</sup>	0.495**	0.438*

Standardized regression coefficients and significance level of a multiple regression analysis are shown

<sup>†</sup>  $p < 0.1$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

<sup>a</sup> Census size not included

**Table 3** Results of the linear mixed-effect models (including and excluding the variable Year) for the effect of temperature and precipitation on census size in 31 *Anacamptis morio* populations in 1977–2010

Year excluded			Year included		
Variable	<i>t</i>	<i>p</i>	Variable	<i>t</i>	<i>p</i>
(Intercept)	7.544	<0.001	(Intercept)	-9.956	<0.001
Temp April	3.981	<0.001	Year	10.300	<0.001
Temp May	-3.598	<0.001	Temp May	-4.430	<0.001
Prec Sep–Oct	3.492	0.001	Temp Dec–Feb	-2.144	0.033
Temp Sep–Oct	2.318	0.021			

temperatures showed a linear temporal increase by 0.8 °C per decade ( $r = 0.586$ ,  $p < 0.001$ ; Fig. 2). Thus, when accounting for this temporal trend by including the main effect *Year* into the model, the positive effect of mean April temperatures on census size was not significant anymore, but the effect of May temperature remained, and additionally a negative effect of high winter temperature was found (Table 3).

#### Population trend

In the long-term dataset, many populations showed increasing census sizes. Population trend  $\mu$  was positive for 23 (74 %) of the 31 populations, which were mainly the large populations (Fig. 3). Population trend was significantly positive for seven populations and significantly negative for one of the 31 populations. The multiple regression analysis of the effects of local site conditions and genetic variation on  $\mu$  in the 19 remnant populations revealed a significantly positive effect of site quality (Table 2).

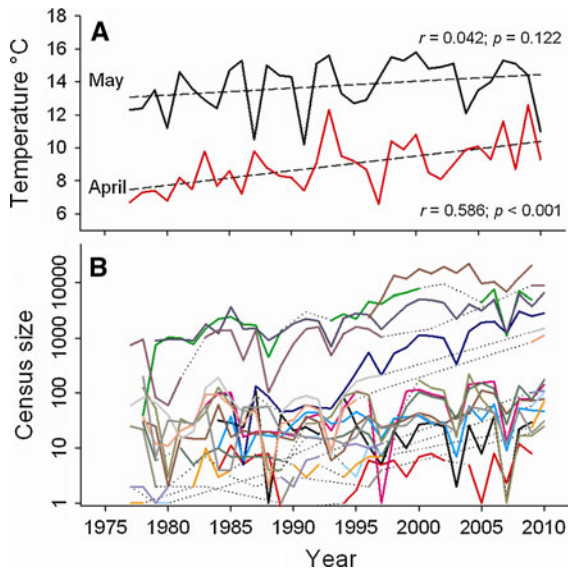
## Discussion

### Effects of site conditions on short-term fitness components

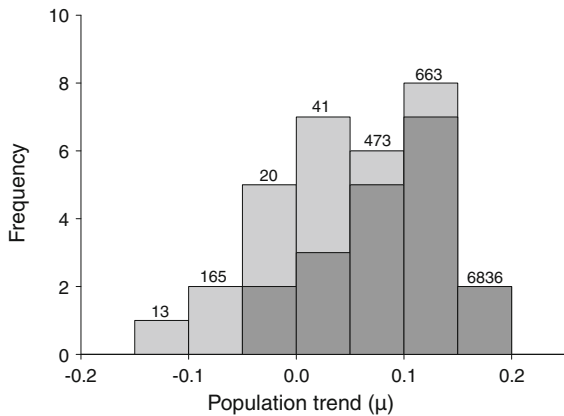
Variation among populations in nearly all components of reproductive fitness was most strongly linked to census size and thus conforms to patterns found in many species (Leimu et al. 2006). Such positive relationships with population size can be attributed to different mechanisms. First, large populations and thus large flowering displays may be more attractive to pollinators and can increase pollinator activity (Ågren 1996). Second, there may be other effects of site conditions (e.g., habitat quality or site heterogeneity) that affect fitness (Leimu 2010; Lauterbach et al. 2011).

Fruit production is known to be low in deceptive orchids due to pollen limitation. Thus, fruit set is usually below 50 %; whereas nectariferous species generally show much higher rates (Neiland and





**Fig. 2** **a** Temporal change 1977–2010 of mean temperature in April and May, **b** population sizes of 19 *Anacamptis morio* populations studied in detail (Table 1). Note that another 12 populations mostly with lower population size and non-positive trend are not shown for clarity. Dotted lines are shown for years without census data



**Fig. 3** Histogram of population trend ( $\mu$ ) from 1977 to 2010 for 31 *Anacamptis morio* populations. Numbers above bars represent mean census sizes within each category. Dark gray bars represent the subset of 19 populations (Table 1)

Wilcock 1998). Hence, our overall estimate of 26 and 32 % fruit set at population and individual level, respectively, fell within the range of reported values in deceptive species and *A. morio* in particular (Neiland and Wilcock 1998; Jersakova et al. 2006; Smithson

2006). However, overall fruit set is the product of shoot survival and fruit set per surviving shoot, and these two were affected differently.

First, shoot survival was positively correlated with site quality index which reflects typical dry grassland attributes like low nitrogen and low moisture. Availability of nutrients can strongly influence growth and reproduction in orchids via interspecific competition (Silvertown et al. 1994) and also water stress has been shown to cause early withering (Vallius et al. 2004). Whether shoot mortality of *A. morio* is enhanced on moisture and more nitrogen rich sites because of biotic interactions like herbivory or pathogens, remains an open question.

Second, and in contrast to the other fitness measures, fruit set of surviving shoots tended to decrease with census size. This finding fits to a hypothesized influence of deceptive pollination on pollinator behavior and its interaction with population size (Jersakova and Kindlmann 1998). In general, pollinators learn to avoid the non-rewarding flowers of deceptive species after some visits (Nilsson 1984). As a consequence, in smaller populations, a larger proportion of flowers may have been visited until avoidance takes place as compared to larger populations. This would result in higher fruit set for the smaller populations. However, avoidance behavior is believed to favor long distance pollen dispersal and may foster outcrossing (Peakall and Beattie 1996), which in turn can increase seed set and quality for larger populations.

Role of genetic variation and connectivity

Despite spatial isolation and substantial variation in population size, most populations showed high values of genetic variation consistent with other studies of long lived, outcrossing species which show a delayed genetic response to habitat fragmentation and habitat loss (Ewers and Didham 2006). Still we found indication of genetic drift. First, a group of spatially close populations had reduced diversity, and therefore may have had a common origin in a putatively bottlenecked ancestral population. Second, there was a weak trend toward a positive correlation between genetic variation and mean census size confirming the general pattern (Leimu et al. 2006). Genetic population differentiation in *A. morio* followed an isolation-by-distance pattern confirming that populations are at

gene flow-drift equilibrium (Hutchison and Templeton 1999) and indicating that despite the dust-like seeds, gene flow by seed is spatially limited.

Deceptive orchids generally show low levels of population differentiation compared to rewarding orchids (Scopece et al. 2010), which is attributed to high outcrossing rates in the former. For *A. morio*, low differentiation ( $G_{ST} = 0.064$  and  $0.055$ ) has been reported in allozyme studies of Italian populations (Scacchi et al. 1990; Rossi et al. 1992). Genetic differentiation in our study ( $F_{ST} = 0.165$ ) was higher than previous estimates but still in the range of other deceptive species (Scopece et al. 2010). More pronounced differentiation in our study region might be attributed to the location at the northern range margin and putatively stronger spatial isolation of populations.

The populations studied clustered genetically into two groups. Group I (Fig. S2) comprised seven, very closely situated populations that showed significant lower genetic diversity than populations of group II. As a consequence, for the complete dataset, the effects of genetic diversity and CI on fitness components could not be separated. However, using a restricted dataset for which genetic diversity and CI were more or less orthogonal, we found a positive effect of CI on fruit set and seed weight. While this finding is well expected (cf. Bizoux et al. 2008), the negative effect of genetic diversity on fruit set is counterintuitive and cannot be explained easily. However, due to the limited data, our finding for CI and genetic diversity should be treated with caution.

#### Short-term weather effects on census size

*Anacamptis morio* populations showed strong annual fluctuations in census sizes which could partly be attributed to weather conditions. High temperature in April, one month prior to flowering, had positive effects whereas high temperature during peak flowering in May had negative effects. Warmer April temperatures may enhance photosynthesis rate thus promoting the development of inflorescences. In contrast, high temperature in May, the main flowering month, may result in water stress on the shallow soils and thus may lead to withering of already developed inflorescences. Our results thus confirm earlier reports of high sensitivity of *A. morio* to spring temperatures (Wells et al. 1998). In addition to flowering and fruit

ripening, bulb formation for the nutrient supply in the next season also takes place in this sensitive phase. Nevertheless, although optimal weather conditions may be important for individual performance, single years of adverse weather may have little long-term effect as dormant individuals may flower in more favorable years (Jersakova et al. 2002).

Winter temperature has been supposed to affect flowering of *A. morio* because of frost damage to winter leaves (Wells et al. 1998). Mild winters on the other hand may enhance flowering as has been found in *Himantoglossum hircinum*, another winter green orchid that reaches its northern distribution range in central Europe (Pfeifer et al. 2006). In contrast, we found that higher winter temperature had either no effect or a negative effect. High winter temperature may be connected with loss of storage carbohydrates due to increased respiration (Bruehlheide and Lieberum 2001).

#### Long-term effects on population trend

Our evaluation of long-term population trend showed that the majority of populations increased since the late 1970s. As discussed above, although changes in the proportion of vegetative to reproductive plants contribute to variation of census size, this should have minor effects when considering long time periods, allowing assessment of changes in population size. The population trend was positive in particular for the large populations. This may be attributed to the fact that the small and decreasing populations are under-represented because they were already extinct. Moreover, if large population sizes result from positive population trend these two measures are not independent of each other. Still, the positive effect of population size on short-term fitness estimates shown above is consistent with the long-term trend. Population trend was also found to increase with site quality. This suggests that nutrient poor and dry conditions, i.e., typical xerothermic grasslands, best fulfill the requirements of *A. morio* both in the short and in the long term. Habitat management should maintain these site conditions to allow for positive population growth. It is known that habitat deterioration is a major reason for decline and extinction of *A. morio* populations. The abandonment of sheep grazing leads to shrub encroachment which is not tolerated by the weak competitor *A. morio* (Silvertown et al. 1994). Also,

nutrient input from adjacent agricultural fields or transformation of meadows into arable land leads to habitat deterioration (Kretzschmar et al. 2007), putatively with more severe effects on population sizes.

Climate change was evident as a strong increase of April temperatures, in line with the general pattern of rising spring temperatures in central Europe, resulting in advanced onset of flowering (Menzel et al. 2001). In *Ophrys sphegodes*, for example, a shift of 6 days per °C rise has been observed within the last four decades (Robbirt et al. 2011). Thus, climate change may drive changes in phenology and subsequently a northward range shift in *A. morio*. However, spring-flowering insect pollinated plants, including *A. morio* are often dependent on a small group of early emerging insects (Nilsson 1984). Although similar shifts have been observed for insect emergence, earlier onset of flowering may lead to a disruption of plant-pollinator interactions with subsequent consequences for reproduction (Bartomeus et al. 2011).

#### Implications for conservation

Overall, our results showed that remnant populations of *A. morio* are mostly demographically stable or increasing. They harbor high levels of genetic variation and reproductive fitness, which are positively affected by nutrient poor and dry habitat conditions. In particular, the findings suggest that climatic change could be beneficial for established populations of *A. morio*. A necessary precondition, however, is the maintenance of typical xerothermic grassland conditions because *A. morio* is intolerant to shade and is outcompeted by larger growing herbs. Climate change may lead to a transformation of semi-dry grasslands which rely to some extent on management to prevent succession to forest-into naturally open xerothermic grassland. It is thus an open question whether historical management like grazing or mowing is still necessary to prevent succession and shrub encroachment. The dust-like orchid seeds may allow colonization of new sites. However, the genetic isolation-by-distance pattern found indicates at least some degree of dispersal limitation. Thus, the likely success of future colonizations will be determined by both, the potential for gene flow (i.e., by seed and pollen dispersal) and the quality of available habitats as a prerequisite for sustainable population sizes and thus, minimized effects of genetic drift.

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