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Neutral and quantitative genetic differentiation among *Trollius europaeus* populations within a fragmented landscape

Andrea R. Pluess · Charlotte Klank · Jaboury Ghazoul

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Abstract In a fragmented landscape, evolutionary processes are expected to differ among small and large remnants of formerly abundant plant species. Genetic drift and/or divergent selection result in population genetic differentiation, while gene flow and/or unifying selection foster genetic similarities. Management strategies for conservation need to consider the (dis)similarities of populations to avoid negative effects of interventions. Quantitative (Q_{ST}) and neutral (F_{ST}) genetic differentiation was investigated in montane populations of *Trollius europaeus*, a plant of wet meadows that has undergone recent habitat loss. We studied plant performance in a greenhouse experiment and estimated genetic variation with AFLPs. By comparing Q_{ST} and F_{ST}, we assessed the importance of selection versus genetic drift among four small, four large and all eight populations. Population genetic variation indicated no loss of diversity in small compared with large populations. Population size classes did not explain the variation of the six measured plant traits. Among the small populations, similar Q_{ST} and F_{ST} estimates in four of the six traits suggested that population differentiation is mainly driven by genetic drift. Among the large populations and across all populations Q_{ST} values were greater than F_{ST} values in four and five of the six traits, respectively, suggesting diversifying selection. Excluding the single high elevation population, however, resulted in Q_{ST}–F_{ST} patterns similar to the small populations. This implies that exchange of genetic material among populations from similar elevations would be a suitable management strategy for maintaining genetic diversity of *T. europaeus* in habitat remnants.

Keywords Cultural landscapes · Habitat fragmentation · Genetic drift · Small vs. large populations · Q_{ST} vs. F_{ST}

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A. R. Pluess (✉) · C. Klank · J. Ghazoul
Ecosystem Management, Department of Environmental Systems Science, Swiss Federal Institute of Technology Zurich (ETH Zurich), Zurich, Switzerland
e-mail: andrea.pluess@env.ethz.ch

Introduction

Effects of land-use change and subsequent fragmentation of natural habitats are often studied in rare and endangered species, yet it is also important to understand effects on species that remain relatively common but which have suffered recent reductions of population sizes and numbers (Honnay and Jacquemyn 2007). Given the global trend of increasing urban sprawl, agricultural intensification and landscape fragmentation (Antrop 1998; McKinney 2006), common species face increasing pressures due to the loss of suitable habitats (Lienert and Fischer 2003; Stehlik et al. 2007). Thereby, individual and population genetic diversity might be lost which is frequently related to reduced individual fitness and population persistence (Reed and Frankham 2003; Leimu et al. 2006; Reynolds et al. 2012). Already small changes in abundance of common species can affect ecosystem structure, function and services to a large degree (Smith and Knapp 2003; Gaston and Fuller 2008). Management might therefore foster also relatively common species through genetic rescue actions. An important prerequisite for conservation practice is the identification of evolutionary units—i.e. populations or groups of populations that are under similar evolutionary processes—in addition to knowledge of the ecological factors affecting

local population persistence (Moritz 1994). An understanding of the processes causing differentiation is important to inform management about the potential usefulness of populations as sources of genetic material for translocation while avoiding genetic swamping effects with potential subsequent outbreeding depression (Frankham 2010).

The two main processes affecting population differentiation are genetic drift and natural selection (Frankham 2005) which might be counterbalanced by gene flow (Lenormand 2002; but see Edelaar and Bolnick 2012). Genetic drift is an arbitrary process whereby random changes in allele frequencies shape the genetic structure of a population, a process more pronounced in small than large populations. Natural selection, on the other hand, is driven by environmental pressures that might vary in a heterogeneous landscape. Both processes shape the distribution of genotypes, which is an important factor affecting a species' adaptive and evolutionary potential (Frankham 1999). While neutral markers such as amplified fragment length polymorphisms (AFLPs) can be used to make inferences on the influence of genetic drift as the cause for population differentiation, detecting differentiation caused by natural selection is more difficult because adaptive genetic markers are rarely available for non-model species and phenotypic traits are commonly under polygenetic control (McKay and Latta 2002). Moreover, phenotypic differentiation in the absence of pronounced selection pressures are shaped primarily by drift effects too.

One approach to differentiate between drift and selection is to compare quantitative (polygenic) genetic variation (Q_{ST}), based on substantially heritable morphological traits, and genetic population differentiation (F_{ST}) derived from neutral molecular markers (Spitze 1993; Merila and Crnokrak 2001). With this approach, the relative roles of natural selection and genetic drift can be assessed and used to determine if plant populations should be treated as separate units or not (Leinonen et al. 2008).

The comparison of Q_{ST} and F_{ST} leads to three principal results (Leinonen et al. 2008). First, if there is no difference between Q_{ST} and F_{ST}, genetic drift and natural selection cannot be distinguished, i.e. the observed differentiation could be achieved by drift alone. Second, if Q_{ST} exceeds F_{ST}, at least part of the population differentiation is caused by diversifying selection favouring different genotypes within different populations. Third, if Q_{ST} is lower than F_{ST}, unifying selection is prevalent. This approach assumes that both differentiation measures have similar rates of drift. Moreover similar mutation rates are expected, an assumption that is difficult to prove (Edelaar and Björklund 2011). While microsatellites have high mutation rates and should therefore not be used in Q_{ST}–F_{ST} comparisons (Edelaar et al. 2011), AFLPs have a lower mutation rate (Kropf et al. 2009) and might therefore serve better to assess the difference of Q_{ST} and F_{ST}.

In this study, we compared quantitative trait differentiation (Q_{ST}) among small and large populations of globeflower (*Trollius europaeus* L. Ranunculaceae) with neutral genetic differentiation (F_{ST}) to evaluate whether populations are subject to diversifying or unifying selection or mainly affected by drift. *T. europaeus* was chosen because the population sizes and numbers have declined across Europe as its wet meadow habitats have been drained for conversion into agricultural land (Muncaciu et al. 2010; Lemke 2011). In our study region in Canton Zurich, northeast Switzerland, the abundance of *T. europaeus* populations has undergone substantial reduction in recent decades, transforming the formerly frequent species into a nowadays rare species (Artendatenbank Canton Zurich, http://www.aln.zh.ch). The remaining individuals are mainly found on nature protection areas and their numbers differ greatly across these sites, ranging from 140 to 820,700 flowers per population in our research sites.

We estimated heritability of the different traits under study with the expectation that these estimates do not differ among population subsets. We then estimated neutral and quantitative genetic population differences with the expectation that the effect of genetic drift is more prevalent in small populations, resulting in similar Q_{ST} and F_{ST} values, whereas large populations could either experience unifying or diversifying selection, depending on whether the population sites are experiencing the same selection forces or not. All but one of the large populations are situated at similar elevations. We therefore re-analysed the dataset excluding the high elevation population and compared these results to those of the overall dataset.

Material and methods

Study species

Characterized by its bright yellow, globose flower, *T. europaeus* L. (Ranunculaceae) is a perennial, hermaphroditic, self-incompatible herb occurring in moist habitats primarily in montane and sub-alpine areas of northern and mid-Europe (Pellmyr 1989; Jaeger and Després 1998; Aeschimann et al. 2004). Up to six fly species of the genus *Chiastocheta* form a highly specialized nursery pollination system with *T. europaeus*, where *Chiastocheta* are the sole pollinators of globeflowers (Pellmyr 1989; Jaeger and Després 1998). Given the small body size of *Chiastocheta* ($\sim$5 mm), pollen dispersal between populations might be limited. Seed dispersal might be limited too, because seed morphology suggests gravity dispersal.

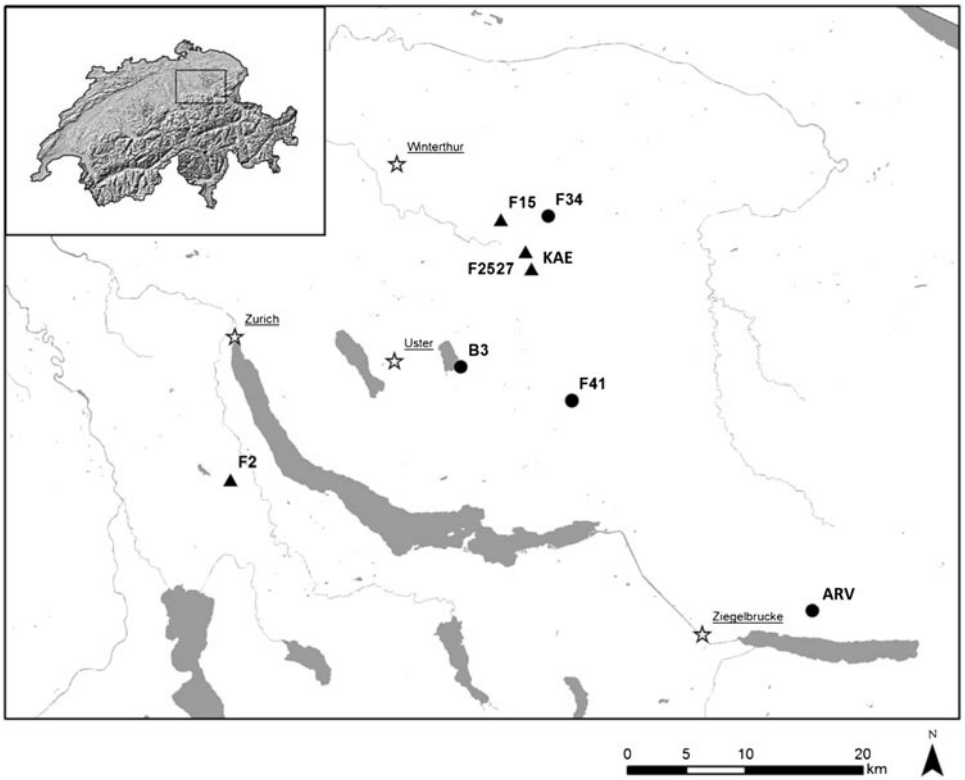

Fig. 1 Location of the eight *Trollius europaeus* populations under study in Switzerland. Large populations are symbolized by a *circle*, small populations by a *triangle*. The *grey areas and lines* are lakes and river systems, respectively. Reproduced with the permission of swisstopo (JA100120)

Table 1 Population characteristics of the *Trollius europaeus* study populations and sample sizes used in the experiments

Pop	Size class	Elev.	Coordinates	Size	Quantitative trait analyses		Neutral genetic analyses		
					N_{SF}	N_{IND}	N_{AFLP}	H_E	PPL
KAE	Small	740	708′454/252′875	140	8	5–10	19	0.208	0.794
F2	Small	745	682′861/235′022	230	8	6–8	22	0.195	0.667
F15	Small	712	705′852/257′047	310	7	7–8	15	0.195	0.635
F2527	Small	612	707′931/254′335	1,120	8	5–9	22	0.179	0.746
F34	Large	597	709′868/257′254	43,360	7	6–8	15	0.172	0.667
B3	Large	537	702′884/244′312	57,780	8	5–7	21	0.162	0.603
F41	Large	752	711′868/241′711	86,460	8	6–8	21	0.211	0.762
ARV	Large	1,250	732′336/223′768	820,700	7	5–8	21	0.213	0.778

Pop population name, *Coordinates* in metres according to the Swiss topographical maps (Bundesamt für Landestopographie, Wabern, Switzerland), *Size* no. of flowers per population, N_{SF}, no. of seed families in the common garden experiment, N_{IND} no. of individuals per seed family, N_{AFLP} no. of individuals for fingerprinting, H_E expected heterozygosity, *PPL* percentage of polymorphic markers

Study sites and plant material

Seed and leaf material was collected from eight *T. europaeus* populations in eastern Switzerland (Fig. 1; Table 1). Population sizes ranged between 140 and 820,700 flowers (see Klank et al. 2010 for a detailed description of the assessment of population sizes). Four of them were classified as "small" with 140 to 1,120 flowers per population and the other four were classified as "large" populations with 43,360 to 820,700 flowers per population. Population sizes differed thereby by a factor 8 and 19 within the small and large population groups, respectively, while large populations were at least 39 times larger than small populations. The distances between populations ranged between 1.57 and 37.64 km, and populations were located between 537 m and 752 m a.s.l., with one population at 1,250 m a.s.l. (Tables 1, 2).

Genetic diversity and differentiation in neutral markers (F_{ST})

To determine population differentiation in neutral marker loci, we used an amplified fragment length polymorphism

Table 2 Geographic distances in km (below diagonal) and pair-wise F_{ST} (above diagonal) for eight *Trollius europaeus* populations

	Small populations				Large populations			
	KAF	F2	F15	F2527	F34	B3	F41	ARV
Small populations								
KAF	–	***0.102***	**0.027**	*0.018*	**0.037**	***0.041***	**0.024**	**0.027**
F2	31.23	–	***0.085***	***0.107***	***0.159***	***0.123***	***0.075***	***0.101***
F15	4.93	31.89	–	*0.023*	***0.081***	***0.042***	*0.027*	**0.039**
F2527	1.57	31.69	3.42	–	***0.043***	0.004	***0.036***	***0.056***
Large populations								
F34	4.71	35.08	4.01	3.58	–	**0.038**	**0.028**	***0.044***
B3	10.30	21.76	13.01	11.27	14.84	–	***0.040***	***0.080***
F41	11.71	29.75	16.53	13.28	15.83	9.86	–	***0.046***
ARV	37.64	50.70	42.54	39.12	40.42	36.40	27.19	–

P values based on permutations ($N = 10{,}098$) of individuals between populations: $P \leq 0.001$, bold, italic and underlined; $P \leq 0.01$, bold and italic; $P \leq 0.05$, bold; $P \leq 0.1$, italic

(AFLP) survey in 15–22 individuals per population (Table 1). Using the three primer pairs *Eco*-ATC/*Mse*-CAG, *Eco*-ATC/*Mse*-CAT and *Eco*-AGA/*Mse*-CTC (Despres et al. 2002) we obtained 54–63 polymorphic loci for analysis, depending on the dataset (all, large, small populations or all and large without the population located at the highest elevation). A detailed description of the protocol and scoring procedure can be found in the Electronic supplementary material (Materials and Methods S1).

DNA fragments of the same length were subsequently expected to be homologous. Linkage disequilibrium (LD) among all pair-wise fragments was tested using Fisher's exact test on contingency tables and corrected for multiple testing using the false discovery approach (FDR, library fdrtool; Strimmer 2008) with the cut-off of FDR(P_i) $\leq$0.05 (Benjamini and Hochberg 1995). LD was significant in 15 of 1,953 comparisons (0.78%). LD, FDR and all other statistics were calculated in R, version 2.10 (R Development Core Team 2009) if not otherwise stated.

The neutrality assumption of the AFLP fragments was tested using BayeScan (Foll and Gaggiotti 2008). No marker showed evidence for being under divergent selection among all populations or between small and large populations, i.e. all markers had a logarithmic Bayes Factor (BF) below 0.8. We conclude, that our dataset conveys the neutrality assumption.

Expected heterozygozity (H_E) was calculated across markers and averaged per population using Arlequin (Excoffier and Lischer 2010). Differences between population pairs were tested with a paired *t* test across markers. Differences of large vs. small populations were tested with a Wilcoxon test on population H_E. Moreover, relation of H_E with elevation of population was tested with a Spearman's rank correlation.

Population differentiation (F_{ST}) was calculated for each dataset averaging marker-based differentiation estimates using a Bayesian approach in BayeScan. To estimate the variation determined by differences between size classes, a hierarchical AMOVA was outlined in Arlequin. Moreover, pair-wise population differentiations were calculated. The pair-wise F_{ST} were then modified according to Rousset (1997) and tested for isolation by distance by relating them to log-transformed geographic distances using GenAlEx (Peakall and Smouse 2012).

Genetic differentiation in quantitative traits (Q_{ST}) and narrow-sense heritability (h^2)

Seeds of 7–8 seed families per population were cold stratified on moist commercial germination soil at 4 °C in the dark for 4 months and then placed in growth cabinets for 10 weeks for germination (MLR-351H, SANYO Electric Co. Ltd.; alternating 12 h light cycles at 15 °C, 50 % relative humidity and 14.4 kLx). Seedlings were then moved into greenhouse compartments and 5–10 individuals of each seed family ($N_{total} = 426$) were transplanted after 2 weeks into 0.5-L pots filled with commercial soil. Plants were then grown under addition of artificial lights (20 kLx) from 8 am to 6 pm and 20/16 °C at daytime and nighttime, respectively. Pot positions were randomized every 2 weeks.

After transplanting the seedlings, we recorded the number of leafs. At the end of the experiment (i.e. after 24 weeks), we recorded the number of leafs, measured stalk lengths of all leafs, leaf area of all leafs and oven-dried above and below ground biomass as fitness proxies. We further calculated the ratio above/below ground biomass and the relative growth rate (RGR) for the total number of leafs using log-transformed values (i.e. $[\log X_2 - \log X_1]/t_2 - t_1$, with *X* being the numbers of leafs at time point *t*) calculated after Hunt (1990). Each trait was analysed for differences between population size class and populations using an

ANOVA. We grouped populations within size class and seed families within populations. A Tukey HSD test was performed to evaluate differences between population pairs.

Assuming a half-sibling design, the narrow-sense heritability was calculated following Petit et al. (2001) as $h^2 = 4V_{FAM}/(4V_{FAM} + V_E)$. V_{FAM} represents the seed family variance component and V_E the residual variance. Q_{ST} were calculated as $Q_{ST} = V_{POP}/(8V_{FAM} + V_{POP})$ where V_{POP} is the population variance component. Variance components were obtained using a fully randomized linear mixed effects model with the REML method and seed families nested within populations as random factors. The 95 % CIs for h^2 and Q_{ST} were obtained by applying a non-parametric bootstrap across seed families with 5,000 iterations using the statistical software R.

To determine the relationship of Q_{ST} and F_{ST}, we estimated the differences (*delta*) of the bootstrap Q_{ST}s with a randomly drawn single marker F_{ST} calculated in BayeScan, analogous to an equivalence test. Using randomly drawn single marker F_{ST}s instead of average F_{ST}s preserves the Chi-square distribution of the F_{ST} estimates (Whitlock 2008). The 95 % CIs of these difference measures (CI_{delta}) indicate if Q_{ST} is bigger (CI_{delta} with a positive range), similar (CI_{delta} including '0') or smaller (CI_{delta} in the negative range) than F_{ST}.

Results

Genetic diversity and differentiation in neutral markers (F_{ST})

Expected heterozygosity (H_E) ranged from 0.16 to 0.21 and percentage of polymorphic loci (PPL) ranged from 0.60 to 0.79 per population (Table 1). H_E was similar among all population pairs ($t \leq 2.5, df = 62, P_{Bonf} > 0.4$). Population level H_E did not differ between large and small populations ($W = 8, P = 1$), but increased with increasing elevation of population origin (rho = 0.958, $P = 0.0002$). PPL did not differ between large and small populations ($W = 8.5$, $P = 0.88$) and tended to be related to elevation (rho = 0.671, $P = 0.07$). The F_{ST} among all, large and small populations were 0.047 (SE = 0.002), 0.044 (SE = 0.0018) and 0.048 (SE = 0.0021), respectively. The hierarchical AMOVA indicated no differentiation between small and large populations ($F_{CT} = 0.0019$, $P = 0.35$). Population pair-wise F_{ST}s ranged from 0.004 to 0.159 ($P < 0.05$ for all but four comparison; Table 2). Population pair-wise F_{ST}s were not related to distance for all and for the large populations ($R^2 = 0.22$, $P = 0.104$ and $R^2 = 0.35$, $P = 0.303$, respectively). In the small population dataset, however, there was a positive relationship due to the population F2, which was farthest away and differed from the other populations with pair-wise F_{ST}s of 0.09–0.11 ($R^2 = 0.92$, $P = 0.022$).

Quantitative traits

While growth traits did not differ between small vs. large populations ($P > 0.1$ for all traits), all traits differed among populations and seed families ($P < 0.03$ for all tests; see Table S1 for trait averages and Table S2 for ANOVA results). For population pair-wise trait comparisons, the Tukey HSD test revealed differences in 45.8 % of comparisons ($P < 0.05$ for those tests; Table S3). The population ARV had the highest frequency of significant trait differences to all other studied populations ($P < 0.02$ for 33 out of 42 comparisons). For pair-wise comparisons between small populations, 13 out of 36 comparisons differed ($P < 0.05$), while for large populations 21 out of 36 were different ($P < 0.05$).

Narrow-sense heritability (h^2) and Q_{ST} vs. F_{ST}

In the full dataset, observed h^2 of individual traits ranged from 0.07 to 0.35 and Q_{ST} ranged from 0.09 to 0.83 (Figure S1 and S2). Large populations had a h^2 of 0–0.36 and Q_{ST} of 0.15–1. If ARV, the population located at highest elevations, was left out h^2 often increased and Q_{ST} was often slightly reduced, but the CIs of the values of the datasets with and without ARV were overlapping. Small populations had a h^2 of 0.11–0.43 and a Q_{ST} of 0.00–0.59. In accordance to the ANOVA results of no differences between size classes, h^2 and Q_{ST} for the two size classes (small and large populations, respectively) were similar as indicated by the overlapping CIs of the respective traits.

Using the *delta* criterion, we found among all, large and small populations that 61 % of the Q_{ST} estimates exceeded the F_{ST} estimates, while the other comparisons did not indicate a difference between Q_{ST} and F_{ST} (Fig. 2). For the dataset using all populations, $Q_{ST} > F_{ST}$ was found for above ground biomass, biomass ratio, leaf area and RGR (i.e. 4 out of 6 traits). For the dataset of large populations, $Q_{ST} > F_{ST}$ was found for above ground biomass, biomass ratio, stalk length, leaf area and RGR (i.e. 5 out of 6 traits). If the population located at the highest elevation (ARV) was excluded from the analyses of all as well as large populations, $Q_{ST} > F_{ST}$ remained for biomass ratio and RGR (i.e. 2 out of 6 traits), while the other traits had similar Q_{ST} and F_{ST} values. Likewise, for the dataset of small populations, $Q_{ST} > F_{ST}$ was found for biomass ratio and RGR only (i.e. 2 out of 6 traits).

Discussion

Across the eight *T. europaeus* populations, Q_{ST} values were frequently larger than F_{ST}, a pattern also prevalent in the

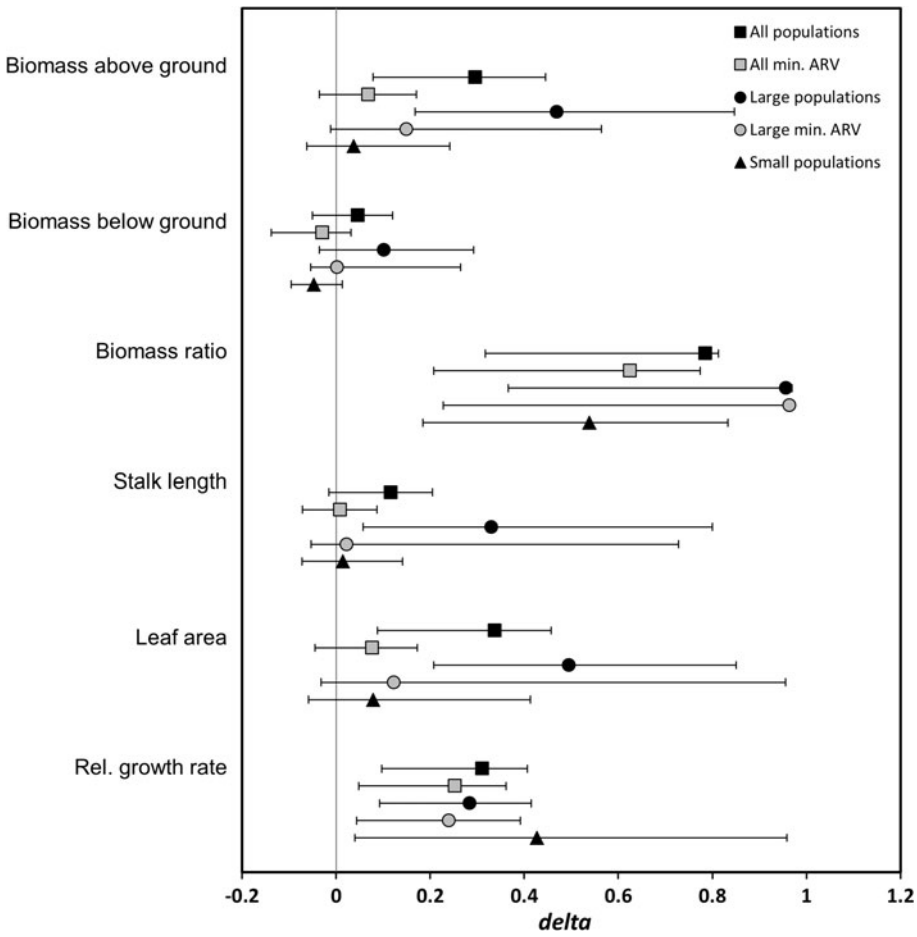

Fig. 2 Q_{ST}–F_{ST} (delta) comparisons of *Trollius europaeus* originating from small or large populations. The dataset of all and large populations were re-analysed after excluding ARV, a population located at higher elevation than the other populations (i.e. All min. ARV; Large min. ARV). *Bars* denote the 95 % confidence intervals (CIs)

large population subset, while the small population subset often had Q_{ST} values similar to F_{ST}. After the exclusion of the higher elevation ARV population the Q_{ST}–F_{ST} patterns for all traits across the remaining seven and the remaining large populations were similar to the patterns found across small populations. The results imply that genetic drift cannot be excluded as the main driver of population differentiation in above- and below ground biomass, as well as average stalk length and total leaf area, in populations located at similar elevations and independent of their size. Biomass ratio and relative growth rate of leaf numbers, on the other hand are under divergent selection in all groups of populations. Likewise, the quantitative genetic differentiation exceeding the neutral genetic differentiation in the datasets including ARV suggests diversifying selection in four and five of the six traits (all and large populations, respectively).

Genetic drift in populations at similar elevations independent of their sizes

Given that no indication for unifying or diversifying selection was found in populations from similar elevations (i.e. excluding ARV) for four traits, random changes in the allele frequencies caused by genetic drift most likely determine the differentiation among large and/or small populations of *T. europaeus*. Generally, large populations are thought to be more resistant to stochastic processes than small populations (Ellstrand and Elam 1993). If so, then divergence due to genetic drift should be less pronounced among large than small populations, as shown in *Ranunculus reptans* (Willi et al. 2007). These populations contained 2 to ca. 500 individuals with 7 out of 12 sites containing fewer than 100 individuals. This is less than the small populations in our study. The 'small populations' of *T. europaeus* might thus not be small enough for a pronounced random loss of alleles which might explain partly why the drift effects were similar among small and large populations.

Drift effects are enhanced if gene flow is low. The relatively low genetic differentiation in our study plant indicates that gene flow occurs even though the pollinators, *Chiastocheta* flies, fly only short distances (Johannesen and Loeschcke 1996; Després 2003) and alternative pollinators play a minor role for successful seed-sets (Ibanez et al. 2009; Klank et al. 2010). The high genetic similarity among populations might also result from the retention of the historical genetic patterns before habitat reduction and fragmentation

occurred (Klank et al. 2012). A marginal isolation by distance effect found among more populations in the same study region by Klank et al. (2012) suggests that gene flow decreases with increasing distance. In contrast, our datasets of all as well as the large populations did not indicate isolation by distance. Yet, the pair-wise differentiation measures are more variable at larger geographic distance and the differentiation increased with distance among small populations due to the population F2, suggesting that random effects can become more important at least between some of the populations located at larger distances (Table 2).

Drift effects are also enhanced if populations are small over long time periods as deleterious mutations become fixed by random processes (Lynch et al. 1995). The resulting drift load was confirmed in long-term small populations of *Arabidopsis lyrata* (Willi et al. 2013). Even though *T. europaeus* population sizes have been reduced over recent decades, the similar heterozygosity of large and small populations indicates that none of these populations have been subject to genetic bottlenecks. Moreover, a lack of ancient genetic bottlenecks in small populations can be assumed, because the species was formerly more frequent in the study area.

Divergent selection in biomass ratio and relative growth rate

All as well as the large and the small population subsets were under divergent selection for the above/below ground biomass ratio and the relative growth rate of leaf numbers. The variation among biomass ratios of seed families within large populations was very small resulting in a quantitative differentiation measure close to one. In both traits, the exclusion of the population from the highest elevation (i.e. ARV) had no qualitative effect on the result. A possible explanation for the pattern found might be the compound nature of the measures. Thus, variation in individual plant traits add up and differentiation among populations might be enhanced. Likewise, in *Hypochoeris radicata* the Q_{ST} of the ratio of vegetative to reproductive biomass was higher than for total above ground biomass or seed mass (0.158 vs. 0.113 and 0.072; Becker 2005). But the effect was reduced for relative growth rates of leaf size in respect to individual leaf sizes in this species (Becker 2005), and in *Scabiosa columbaria*, Q_{ST} of the relative growth rate of length of the longest leaf was zero (Scheepens et al. 2010), indicating, that combining plant traits might not necessarily enhance quantitative genetic differentiation.

Overall, an indication for diversifying selection is a conservative finding, because several effects which might bias Q_{ST} estimates such as dominance, epistasis and non-additive components, lower the Q_{ST} estimate relative to the F_{ST} (Lopéz-Fanjul et al. 2003; Goudet and Büchi 2006). This might also influence the lack of divergent selection reported for several traits above. Nevertheless, we assume that the potential downward bias of Q_{ST} is probably negligible in our Q_{ST}–F_{ST} comparison, mainly because differentiation of neutral genes was low.

Potential effect of elevation on selection

Because the indications for divergent selection in most traits disappeared after the exclusion of the single high elevation population ARV, the selection pattern found might be likely due to differences in elevational population origin. Diversifying selection can be found along environmental gradients resulting into local adaptation (Leimu and Fischer 2008). In *Rutidosis leptorrhynchoides*, for example, phenotypic divergence could be explained by the environmental distance determined by differences in soil, climate and elevation (Pickup et al. 2012). A proxy for the environmental differences among populations in our study is the elevational origin. But given that only one population (ARV) was from higher elevation, we cannot draw firm conclusions on the effect of elevation. Interestingly, individuals reared from ARV had the lowest mean values for almost all traits measured in the experiment (Table S1). The greenhouse conditions were probably closer to the field conditions of the populations at lower elevations. This combined with the finding that growth of plants transplanted to lower elevations can be reduced in altitude-adapted plants (Clausen et al. 1941), our results might indeed suggest that elevational origin might have an effect on quantitative genetic population differentiation.

Conclusion for conservation of small populations

Given that ecotypic variation can play an important role in conservation management when dealing with habitat restoration or reinforcing populations (McKay and Latta 2002; Vergeer et al. 2004), information on the suitability of plants of non-local origin is important. To circumvent potential outbreeding depression through artificial combination of distinct populations (Tallmon et al. 2004), populations with similar measures for ecological relevant traits should be used. A further conservation concern is the level of genetic diversity (Ellstrand and Elam 1993; Schemske et al. 1994; Leimu et al. 2010), which in our study was similar across the populations of different sizes, indicating that small populations are not genetically depleted and generally suitable as seed sources for management schemes. Neutral genetic differentiation was low and no selection patterns were detected for all but the compound traits in populations of similar elevation suggesting that these populations form a potential conservation unit within our study region.

Conservation action should take place before the consequences of isolation and fragmentation becomes of concern, because subsequent fitness changes can be a slow process (Lopez et al. 2009). We conclude that promoting the addition of genetic material to small populations to augment population sizes and to support the preservation of the genetic diversity would be a suitable conservation strategy for maintaining *Trollius europaeus* in small nature reserves, given that plant material originates from similar elevations.

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