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From the bare minimum: genetics and selection in populations founded by only a few parents

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ABSTRACT

Question: Genetic variation is expected to control the fate of populations colonizing new environments, because the amount and nature of this variation influences adaptation. Thus, it is generally expected that the ability of populations to colonize new environments is severely compromised if the number of founding individuals is very few.

Organisms: Brown trout (*Salmo trutta*) are native to the northern hemisphere, but have been widely introduced globally, including into the southern hemisphere.

Times and places: We analysed two isolated populations of brown trout introduced in 1993 to the remote Kerguelen Islands in the sub-Antarctic region, each population being founded with the offspring of only a single mother, and with either only one or two fathers.

Methods: Scale samples were collected in 2003 and 2010 and analysed using a set of 16 microsatellite markers. These data were used to calculate individual homozygosity level and variance in inbreeding. The association between age (estimated through scale reading) and homozygosity level was used to assess the potential consequences of low genetic variation for individual survival.

Results: The two populations represented different outcomes. In one population, variance in inbreeding was high and a clear heterozygosity–fitness correlation (HFC) was evident: older individuals were less homozygous than younger individuals. Consistent with these results, homozygosity level in this population decreased from 2003 to 2010. In the other population, variance in inbreeding was low, no consistent HFC was detected, and no decline in homozygosity level was evident from 2003 to 2010. Low genetic variation and severe initial bottlenecks through founding effects did not prevent the establishment and success of these populations, one of which appears to be actively purging inbred individuals.

Keywords: genetic variation, heterozygosity, inbreeding, invasion, small population.

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INTRODUCTION

Genetic variation is the fuel for natural selection, and thus is expected to play a major role in both large and small evolutionary events by increasing the rate and efficacy of adaptation. At the intra-locus scale, high allelic diversity should favour adaptation by increasing the variance in fitness and by increasing the chance that at least some variants are beneficial (Caballero and Garcia-Dorado, 2013). At the inter-locus scale, positive effects of high allelic diversity should come from the breakdown of linkage disequilibrium and the corresponding construction of new allelic combinations (Keller *et al.*, 2014). These benefits of genetic diversity are expected to be critical in the colonization of new environments, a situation where rapid adaptation should be particularly important (Baker and Stebbins, 1965; Barrett, 2015). For instance, biologists studying invasions often relate colonization success to the initial number of founders, the so-called 'propagule pressure' (Olden *et al.*, 2010; Blackburn *et al.*, 2011; Mimura *et al.*, 2013). Although part of this effect is demographic, a number of studies have also highlighted genetic contributions. For instance, some successful invasions appear to have been boosted by multiple invasion waves and multiple origins that increase genetic variation (Calsbeek *et al.*, 2011).

In contrast, populations founded by small numbers of individuals of a single origin should be more prone to extinction or should at least show slower adaptation. The numerous genetic problems (in addition to difficulties in adaptation as implied above) that such populations face include reduced allelic diversity, strong genetic drift, difficulty in generating new linkage combinations, and inbreeding that can expose deleterious alleles (Charlesworth and Charlesworth, 1987; Frankham, 1995). The relationship between high inbreeding and the expression of recessive deleterious alleles is of particular interest because the outcome is expected to be strong selection against homozygosity. A number of studies support these expectations (Cain *et al.*, 2014; Forcada and Hoffman, 2014; Haanes *et al.*, 2014); yet others have shown that reduced genetic variation does not always prevent invasion (Puillandre *et al.*, 2008) or adaptation (Kaeuffer *et al.*, 2008; Rollins *et al.*, 2013; Wood *et al.*, 2015). One of several suggested solutions to this apparent paradox is the conversion of epistatic or dominance genetic variance to additive genetic variance for fitness traits (Lindholm *et al.*, 2005). Most studies considering these options have adopted retrospective approaches, using present DNA variation to infer past events, such as bottlenecks. Yet it should be more directly informative to track the fate of multiple generations of new populations following their founding with reduced genetic variation (Britton and Gozlan, 2013), which was the goal of our study.

The invasion of the Kerguelen Islands by brown trout (*Salmo trutta* L.) involved multiple introduction attempts (from 1954 to 1978) from multiple geographic and genetic origins (wild and hatchery fish from France, Poland, and Denmark). These fish spread from 1962 to 2010 to colonize the entire eastern half of the archipelago (Launey *et al.*, 2010; Lecomte *et al.*, 2013). In the western half, however, the invasion has been slower, perhaps due to novel ecological conditions that restrict natural colonization and adaptation (Labonne *et al.*, 2013). Instead, the two westernmost populations in Kerguelen (Val Travers and Clarée) were both artificially introduced in 1993. These populations were founded by the offspring of only two (Val Travers) or three (Clarée) parents sampled in other rivers of Kerguelen. The populations continued to persist three to four generations after the introduction and they remain geographically and genetically isolated from other Kerguelen populations (Labonne *et al.*, 2013). This situation provides an excellent opportunity to study genetics and selection in small populations encountering new environments.

We describe genetic variation currently present in the two populations and ask how selection shapes that variation. We specifically focus on heterozygosity–fitness correlations (HFC). These correlations rely on inbreeding variation within populations and relate a fitness proxy (e.g. survival or reproductive success) to individual heterozygosity (Bennett and Binet, 1956; Weir and Cockerham, 1973; Chapman *et al.*, 2009; Szulkin *et al.*, 2010). Using a set of 16 microsatellite markers, we (1) estimate the individual inbreeding coefficient and its variance within each population, (2) compute individual homozygosity levels (Aparicio *et al.*, 2006), and (3) investigate if individual fitness (age at capture as a proxy) is related to within-individual homozygosity. We then interpret similarities and differences between the two populations with regard to environmental context and inbreeding dynamics.

METHODS

Populations and sampling

The Val Travers system (49°18′32″S, 69°25′79″E) is 9 km long with a gradient of habitats and slopes from mountain to lowland landscapes. It empties into Lake Bontemps (700 ha), which is connected to the sea by a steep outlet that trout cannot ascend. The Clarée system (49°29′35″S, 69°37′44″E) is 3 km long and is situated on a plain featuring several interconnected arms originating from Lake Hermance (350 ha) and a tributary from a nearby glacier (River Galets). It empties into the open sea. The trout populations in both rivers were artificially introduced in 1993 from two other Kerguelen rivers (Lecomte *et al.*, 2013). The Val Travers population was founded with 2000 fry from a single cross between one male and one female from the River Chateau, which was first colonized in 1962. The Clarée population was founded with 1700 fry from a cross between one female and two males that were captured while migrating upstream in the River Armor, which at that time was not yet colonized by brown trout [no natural reproduction observed (Lecomte *et al.*, 2013)] (see Fig. 1).

In 2003 and 2010, both populations were sampled using electrofishing and hook-and-line for a total of 332 individuals: 103 in 2003 and 94 in 2010 for Val Travers, and 42 in 2003 and 93 in 2010 for Clarée. The captured fish were anaesthetized using phenoxy-ethanol, measured for total length, and scales and fin tissue were collected. The scales were kept in paper envelopes protected from moisture, and the fin tissue was placed in 90% ethanol. Individual age at capture was determined by scale reading, which was validated by individually tagging fish in 2010 that were recaptured in 2011 or 2012. When age could not be determined with confidence (due to regenerated scales, $N = 77$ fish), a Von Bertalanffy model was used to estimate age from total length (see www.evolutionary-ecology.com/data/2970Appendix.pdf). In three designated areas for each population in 2010, 2011, and 2012, a De Lury sampling protocol was applied to estimate local densities for fish older than 2 years. Average densities were 276 and 387 fish per hectare for Val Travers and Clarée respectively. Population size (obtained by generalizing these numbers to the average available river area) was similar for Val Travers and Clarée, with about 2300–2400 fish in each population.

Genotyping

DNA was extracted with a modified NaCl/chloroform-based protocol (Müllenbach *et al.*, 1989). Sixteen microsatellites previously developed for salmonids were amplified: SsaD190 (King *et al.*, 2005); SSOSL438 (Slettan *et al.*, 1996); Ssa103NVH, Ss4, Str 58, SsaT47Lee,

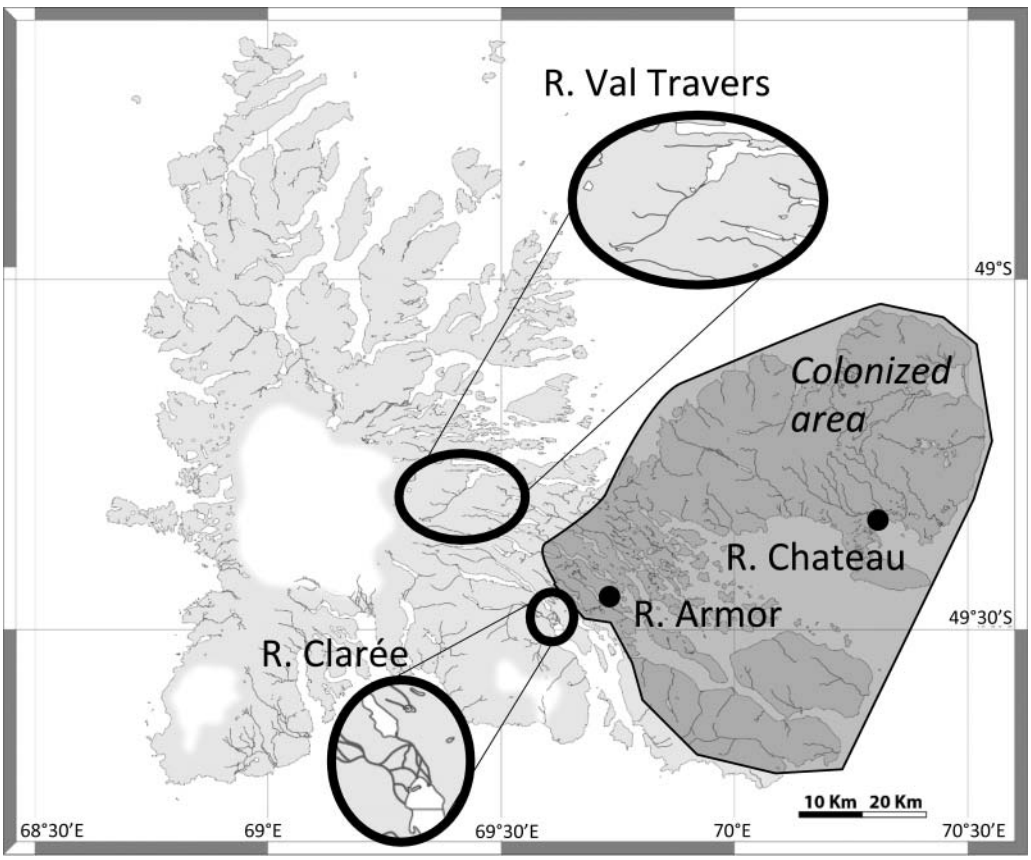

Fig. 1. Locations of the study populations (ovals) and their populations of origin (solid circles) in the eastern part of the Kerguelen archipelago. Grey shading indicates the area previously colonized by brown trout.

Ssa121NVH, Ssa179NVH, Ssa159NVH, T3-13 (Gharbi *et al.*, 2006); SSOSL85, SSOSL311, SSOSL417 (Slettan *et al.*, 1995); StrUBA (Coughlan *et al.*, 2006); Ssa197 (O'Reilly *et al.*, 1996); and OmyRT5U (Gharbi *et al.*, 2006). All of these markers are on separate linkage groups except the last two, which are both located on BT-28 (Gharbi *et al.*, 2006) (2970Appendix.pdf). Amplification was by multiplex polymerase chain reaction (2970Appendix.pdf).

The PCR products were sized on an ABI Prism 3100-Avant sequencer (Life Technologies) using a GeneScan 500 LIZ internal size standard (Life Technologies). Fragments were scored and raw allele sizes were binned into discrete allele classes using Genemapper software (Life Technologies). When an allele was found only once in the dataset (i.e. a unique allele), it was considered mistyped. A second analysis was run for samples presenting more than two null or mistyped loci. When samples still showed a unique allele in the dataset, they were considered to be null for the locus in question (coded as non-available). Only four Val Travers fish and one Clarée fish could not be successfully genotyped.

Samples were genetically sexed by PCR with the Yanng176FR marker linked to the sex control region (Yano *et al.*, 2012, 2013). We also used a control for amplification derived from the eye-specific lactate dehydrogenase sequence gene (GenBank accession numbers

AF 488539 and AJ277710), which generates an amplification product of approximately 1 kb (Régnier *et al.*, submitted). The PCR protocol can be found at 2970Appendix.pdf.

Statistical analyses

Hardy-Weinberg equilibrium was tested for each locus in each population using Cervus 3.0 (Kalinowski *et al.*, 2007) and across-locus F_{IS} was calculated using Genetix 4.02 (http:kimura.univ-montp2.fr/genetix/). Also for each population, the mean inbreeding coefficient (f) and effective size (N_e) were estimated using Colony 2.0 [non-random mating scenario (Jones and Wang, 2010)]. Identity disequilibrium (ID) based on g_2, a two-locus heterozygosity disequilibrium estimate over all pairs of loci, was estimated for each population with the RMES software (David *et al.*, 2007). Homozygosity level [HL (Aparicio *et al.*, 2006)] was calculated for each individual. We then tested for a decrease in HL between 2003 and 2010 by means of a one-sided Wilcoxon rank sum test on individual HL. Finally, we tested the homozygosity–fitness correlation (HFC) by relating HL to the age of individuals using a quasi-Poisson generalized linear model. This model also accounted for effects of sex and sampling year (2003 or 2010) as fixed categorical factors. Age at capture was assumed to be positively related to fitness, under the expectation that older individuals represent a subset of their cohort with traits conveying better survival and thus having more opportunities to reproduce. Additionally, age at maturity in Kerguelen is reached at 4–5 years of age. Thus, lower HL in older individuals should indicate selection against homozygosity. A separate model was fit for each population.

RESULTS

Genetic variation and inbreeding

Only two loci showed departures from Hardy-Weinberg equilibrium (2970Appendix.pdf). STRUBA showed a significant excess of homozygotes in both populations, and SSOSL438 showed a significant excess of homozygotes in Val Travers only. Consistent with these results, estimates of null allele frequency were high for both STRUBA (0.1743 in Clarée and 0.2649 in Val Travers) and SSOSL438 (0.2210 in Val Travers). Mean allelic diversity was 4.875 for Val Travers and 6.437 for Clarée. F_{IS} values were 0.04432 (95%CI: 0.02512–0.05890) for Clarée and 0.05856 (95%CI: 0.02887–0.06791) for Val Travers. The inbreeding coefficient was relatively high in both populations, although more so in Val Travers ($f = 0.0647$) than in Clarée ($f = 0.0364$; Table 1). Correspondingly, the effective population

Table 1. Summary of inbreeding parameters

Parameter	Val Travers	Clarée
N_a	4.875	6.4375
f	0.0647	0.0364
g_2	**0.0199***	0.0018
N_e	12 [6–26]	46 [30–72]

Note: N_a is the mean allelic diversity, f is the inbreeding coefficient, g_2 is the identity disequilibrium, and N_e is the effective population size. For the g_2 parameter, bold font indicates values significantly different from zero.

Table 2. Deviance analysis for the quasi-Poisson generalized linear model for each population

Factor	d.f.	Deviance	*F*-statistic	*P*-value
Val Travers				
Homozygosity level (HL)	1	10.6345	9.1604	**0.002863**
Sex	1	4.4017	3.7915	0.053180
Year	1	0.4304	0.3708	0.543417
HL × Sex	1	1.4633	1.2605	0.263165
HL × Year	1	0.7297	0.6285	0.429013
Sex × Year	1	0.0019	0.0016	0.967757
HL × Sex × Year	1	0.0843	0.0726	0.787855
Residuals	168	180.92		
Clarée				
Homozygosity level (HL)	1	2.5747	1.7226	0.191943
Sex	1	4.1693	2.7896	0.097575
Year	1	20.0924	13.4433	**0.000372**
HL × Sex	1	1.4705	0.9839	0.323312
HL × Year	1	0.1738	0.1163	0.733735
Sex × Year	1	0.3898	0.2608	0.610558
HL × Sex × Year	1	6.2199	4.1616	**0.043622**
Residuals	116	146.60		

Note: The significance threshold α has been set to 0.05.

size (N_e) was lower in Val Travers ($N_e = 12$) than in Claree ($N_e = 46$). Identity disequilibrium was high in Val Travers [$g_2 = 0.0199$, $p(g_2 = 0) = 0$] but not Clarée ($g_2 = 0.004$, $p(g_2 = 0) = 0.107$; Table 2). Individual HL varied from 0.10 to 0.79 in Val Travers (mean = 0.392) and from 0.046 to 0.68 in Clarée (mean = 0.272) (Fig. 2). A significant decrease in HL between 2003 and 2010 was detected in Val Travers ($P = 0.001696$, Wilcoxon rank sum test), but not in Clarée ($P = 0.9075$, Wilcoxon rank sum test).

Homozygosity–fitness correlation

Homozygosity–fitness correlations were tested using 15 microsatellite markers, excluding the STRUBA locus, which is known to generate frequent null alleles. Quasi-Poisson models revealed a strong HFC in Val Travers (Table 2), wherein older individuals had lower homozygosity than did younger individuals (Fig. 3A). In Clarée, a strong effect of year on age at capture was detected (Table 2), perhaps due simply to variation between years in the average age of the sampled fish. Also in Clarée, a three-way interaction was weakly significant: the effect of homozygosity level on age at capture was influenced by year and sex. Specifically, the model predicted that, in 2010, homozygosity level had no effect on age at capture but, in 2003, older females as well as younger males had higher homozygosity (Fig. 3B). Similar results were obtained when removing the SSOSL438 locus for which Hardy-Weinberg equilibrium was not satisfied in one population.

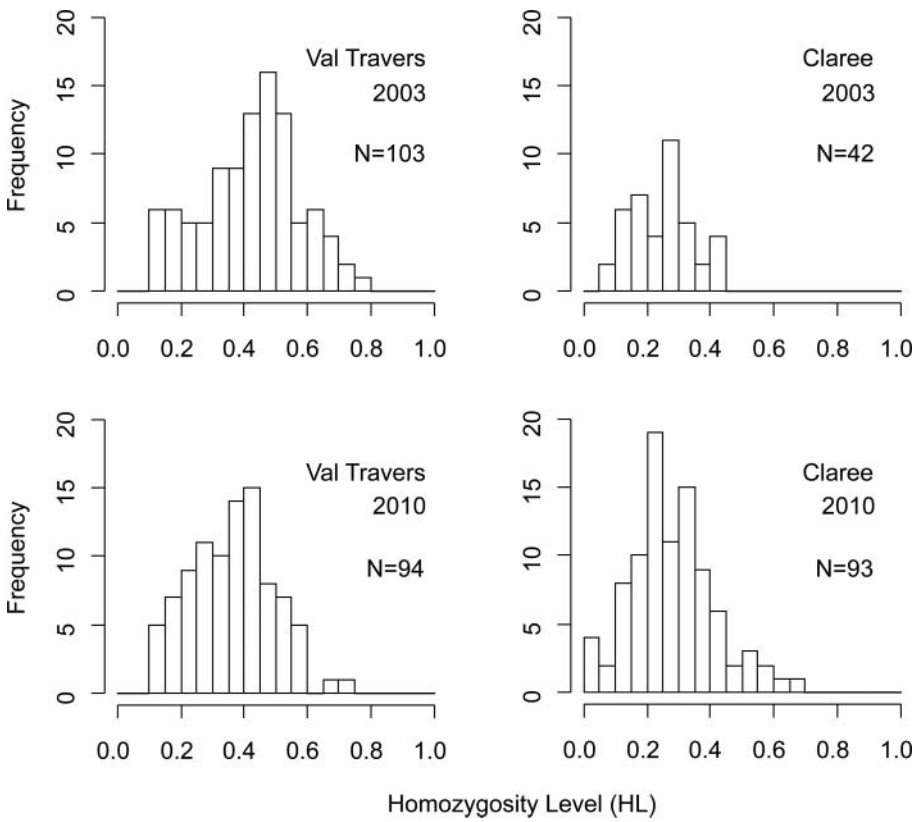

Fig. 2. Distribution of homozygosity level (HL) in Val Travers and Clarée for the two sampling years (2003 and 2010).

DISCUSSION

As expected from the very few initial crosses that founded each population, a high level of inbreeding was detected. Also in line with expectations, inbreeding was higher and allelic diversity was lower in Val Travers, founded by a cross of one female and one male, than in Clarée, founded by one female and two males. And yet, surprisingly, the majority of loci were in Hardy-Weinberg equilibrium, and allelic diversity was relatively high in both populations (up to 10 alleles in Clarée and 8 in Val Travers; 2970Appendix.pdf). Beyond these broad similarities, the populations differed for variance in inbreeding (as measured by identity disequilibrium), with essentially no variance in Clarée but high variance in Val Travers. Indeed, the variance in Val Travers was high even in relation to other taxa that are not known to have recently gone through a major bottleneck (Chapman *et al.*, 2009; Miller and Coltman, 2014). For heterozygosity–fitness correlations, less homozygous individuals in Val Travers had an older age at capture, suggesting viability selection against homozygosity. In Clarée, HFCs were much weaker and varied between the sexes and sampling years. Consistent with these differences in selection, homozygosity level decreased in Val Travers between 2003 and 2010, whereas it remained stable in Clarée over the same period.

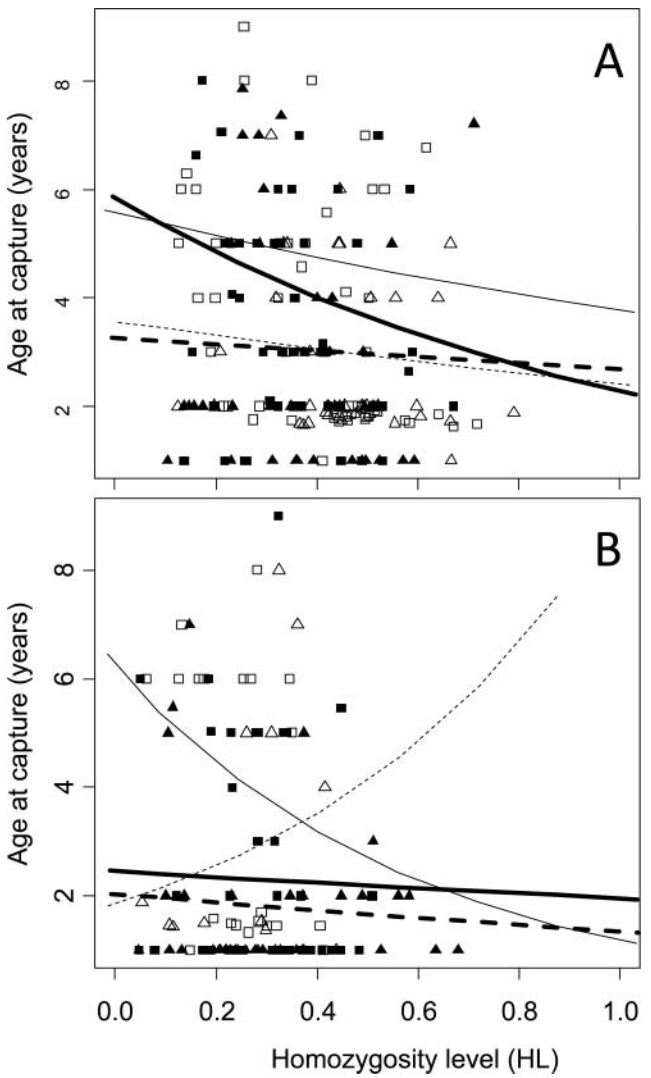

Fig. 3. Effect of homozygosity level (HL) on age at capture for the Val Travers (A) and Clarée (B) populations. Open symbols indicate data sampled in 2003 and solid symbols indicate data sampled in 2010 (triangles for females, squares for males). Curves show predictions from the quasi-Poisson generalized linear model (full lines for males, dashed lines for females; thin lines indicate 2003 predictions and bold lines indicate 2010 predictions).

Genetic variation

Allelic diversity in both populations was surprisingly high given the strong founding events. Without mutation or immigration, the maximum number of alleles would have been 4 and 6 in these two populations, whereas half of our markers (often the same markers in both populations) had more alleles. Several hypotheses might explain this paradox: genotyping error, immigration, misinformation, and mutation. Genotyping error can be ruled out

because, when genotyping 64 individuals twice, we found discrepancies for only two markers: SSOSL438 (error rate = 0.0625) and SSOSL85 (error rate = 0.03125). Immigration also can be ruled out for Val Travers, which is isolated by an impassable barrier and, indeed, no sea trout have ever been found (J.C. Aymes, unpublished data). For Clarée, immigration is possible but unlikely because the nearest colonized river (Levant) is very far away [55.3 km shoreline distance (Labonne *et al.*, 2013)], and anyway was founded with the same initial cross as Clarée. Moreover, the Clarée population did not have a greater discrepancy between expected and observed allele numbers than did Val Travers. Misinformation about the number of founders also seems unlikely given the precise notes taken at the time.

Discounting the above possibilities – at least to the extent possible – leaves the role of new mutations. Mutation rates for microsatellite markers can be very high [up to 8.5×10^{-3} (Steinberg *et al.*, 2002)], and thus might generate the observed results, particularly if heterozygous individuals are favoured, as our data for Val Travers show. Indeed, a simple demogenetic simulation using two founders set up to mimic our situation (high demographic growth rate with high fecundity and high initial survival) indicates that a rapid increase in allelic variation for neutral markers could indeed be observed for mutation rates ranging from 1×10^{-4} to 1×10^{-3} (2970Appendix.pdf). More specifically, our data would suggest a mutation rate between 5×10^{-4} and 1×10^{-4}, which is a very likely range for salmonids (Steinberg *et al.*, 2002).

How does the current allelic diversity compare with other brown trout populations? Native European populations generally show slightly higher allelic diversity, with often the same markers providing higher numbers of alleles (Hansen *et al.*, 2009; Horreo *et al.*, 2011). Allelic diversity estimates for other naturalized populations in the southern hemisphere can sometimes be lower than in European populations, but they encompass our present results (Valiente *et al.*, 2007; Monzon-Arguello *et al.*, 2014). In Kerguelen, allelic diversity in other populations can be rather high [up to 9.1 (Launey *et al.*, 2010)]. This is especially the case in rivers where introductions happened first (River Chateau, River Studer, and River Nord), possibly as a result of multiple origins in the founding stock (Lecomte *et al.*, 2013). Natural colonizations of nearby rivers from these initial source populations led to the recent foundation of new populations that generally also have high mean allelic diversity (range 5.1–8.3), often above those found for Val Travers and Clarée. Interestingly, F_{IS} estimates for these naturally founded populations encompass what we found for Val Travers and Clarée and can sometimes be even higher [up to 0.083 (Launey *et al.*, 2010)], indicating that it is possible for some populations to retain high inbreeding levels despite persisting longer than Val Travers or Clarée. This could occur if such populations had smaller effective population sizes or experienced repeated bottlenecks. It therefore seems that populations in Kerguelen do not present a strongly reduced allelic diversity – compared with European populations or other invasive populations – and that this allelic diversity can be quickly restored following introduction or colonization bottlenecks, despite presenting relatively high inbreeding levels.

Inbreeding

Although inbreeding was relatively high, it might have been even higher given the few founders and the few subsequent generations. In Val Travers, for instance, the minimal expected inbreeding coefficient calculated using a simple pedigree approach (assuming individuals will always choose the more genetically distant mate) is about 0.164 at the third

generation. Similar values were reported using a pedigree approach in a moose population by Haanes *et al.* (2014). The discrepancy between our high expected inbreeding value (0.164) and the much lower observed inbreeding values (0.0647 and 0.0364) might again be explained by the relatively high mutation rate of microsatellites, as discussed above. Additionally, estimates of inbreeding via identity disequilibrium might not always capture the full extent of inbreeding variation (Grueber *et al.*, 2011).

Of further interest, the two populations showed a large difference in inbreeding variance, as measured by the g_2 parameter (David *et al.*, 2007). Variance in inbreeding within a population can arise through genetic drift and bottlenecks, two mechanisms with stochastic outcomes that are expected to be strong in such small populations (Bierne *et al.*, 2000; Lenormand *et al.*, 2009; Grueber *et al.*, 2011). Another potential driver of variance in inbreeding is the structure of mating systems (Weir *et al.*, 1980), which might suggest that mating systems differ between the two populations. Although no such difference has been identified, it would certainly be consistent with the lower effective population size in Val Travers (Table 1). That is, mating systems biased towards monogamy (i.e. reduced operational sex ratio) and with a small effective size tend to generate higher variance in inbreeding (Weir *et al.*, 1980).

It is not clear to what extent brown trout avoid mating with close kin. Such avoidance is at most conditional and not prohibitively strong, since both populations in this study were initially founded by kin that must have mated and done so extensively to colonize these systems so quickly. However, we might look for a signal of such inbreeding avoidance effects at the StrUBA locus, which is linked to the MHC-1 complex involved in pathogen resistance in salmonids (Coughlan *et al.*, 2006; Forsberg *et al.*, 2007). The MHC complex is thought to be under sexual selection in many vertebrates, wherein females can select more or less dissimilar genotypes with respect to their own genotype (Wedekind *et al.*, 1995; Jacob *et al.*, 2010). However, both populations showed approximately the same level of excess of homozygotes at the StrUBA locus (2970Appendix.pdf), suggesting they have similar, if any, patterns of kin avoidance. In the case of Kerguelen, however, no pathogens of brown trout have been detected, suggesting that this locus could be undergoing relaxed natural selection (i.e. the advantage of being heterozygous could be non-existent).

Homozygosity–fitness correlations

The detection of strong HFCs in small populations is not very common (Chapman *et al.*, 2009; Grueber *et al.*, 2011), and it is even more rare to actually monitor the dynamics of HFCs since a population's founding (but see Haanes *et al.*, 2014). In this context, our results reveal that selection against homozygosity can be active when genetic variation is initially minimal and populations are small and in new environments. Similar results have been found in other studies (Kaeuffer *et al.*, 2007; Puillandre *et al.*, 2008), indicating that small population sizes do not preclude the action of selection. Indeed, it seems that small populations undergo active selection for increased variation.

Yet any attempt at generality must be tempered by the reality that HFCs differed between the two populations. In Val Travers, homozygous individuals (HL = 0.75) are rare among older fish, both in the first few generations post founding (2003) and also some years later (2010). Given that most of the fish in these populations are mature at 4 or 5 years of age, this selection against homozygosity would increase genetic variation in the next generation. In Clarée, such selection against homozygous individuals was weaker and inconsistent,

being found only for males sampled in 2003. Several potential explanations can be proposed for this difference between the populations. First, the fact that HFCs are weaker or non-existent in Clarée might be directly related to the lack of variance in inbreeding in that population: all individuals are similarly inbred, meaning that fitness differences due to inbreeding load will be less likely to arise. An alternative is that the inbreeding level is not related to fitness variation, simply because no deleterious mutations are present in this population, or because low genetic variation does not affect individual fitness in this environment (Valiente *et al.*, 2010). Finally, it is possible that the purging of deleterious alleles happened very early, which would be consistent with the disappearance of the correlation in males from 2003 to 2010. Yet this last hypothesis requires an explanation as to why males but not females show selection against homozygotes.

Another class of explanations for the population differences in HFCs is environmental differences. One environmental difference relates to diet, with young fish in Clarée able to feed on abundant zooplankton from an upstream lake (Wojtenka and Van Steenberghe, 1982; personal observation). By contrast, Val Travers does not have an upstream lake and so stream juveniles feed mainly on terrestrial prey, which are relatively rare in Kerguelen. Another striking difference between the two populations is the accessibility of marine migration: potentially easy in Clarée but impossible in Val Travers. Thus, associations between homozygosity and migration could cause population differences. Note, however, that differences in immigration from the marine environment cannot explain the differences in HFCs because neither population experiences immigration (see above).

Finally, the origin of founders might play a central role. In the present case, the founders of the Val Travers population were obtained from an already established population, indeed the oldest known population in Kerguelen (River Chateau). They were therefore resident fish in that system, and possibly already related. The founders of Clarée, by contrast, were caught during an upstream migration in a system (River Amor) where brown trout reproduction had not yet occurred. They were therefore migratory fish, pioneering new environments, and they may have originated from different rivers. Disentangling all of the above alternatives will require further work.

CONCLUSION

About 40 other rivers have been successfully colonized by brown trout in Kerguelen, and no local extinction has been documented since the first successful introduction in the archipelago (Labonne *et al.*, 2013). Our results show that, with minimal genetic variation and very strong founder effects, two 'replicate' introductions can yield different genetic outcomes – with one population showing high variance in inbreeding and a small effective size, and the second population showing barely any variance in inbreeding and a rather high effective size. Moreover, heterozygosity–fitness correlations were strong and stable in one population but not in the other. Thus, selection is currently working to increase genetic variation in one population and may already have done so in the other populations. Although strong founder effects are expected to be problematic for organisms colonizing new environments, our results show that such circumstances do not preclude success and that selection might actively work to overcome the initial genetic disadvantages of a small founding gene pool.

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