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Are we underestimating the occurrence of sympatric populations?

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Abstract

Sympatric populations are conspecific populations that coexist spatially. They are of interest in evolutionary biology by representing the potential first steps of sympatric speciation and are important to identify and monitor in conservation management. Reviewing the literature pertaining to sympatric populations, we find that most cases of sympatry appear coupled to phenotypic divergence, implying ease of detection. In comparison, phenotypically cryptic, sympatric populations seem rarely documented. We explore the statistical power for detecting population mixtures from genetic marker data, using commonly applied tests for heterozygote deficiency (i.e., Wahlund effect) and the STRUCTURE software, through computer simulations. We find that both tests are efficient at detecting population mixture only when genetic differentiation is high, sample size and number of genetic markers are reasonable and the sympatric populations happen to occur in similar proportions in the sample. We present an approximate expression based on these experimental factors for the lower limit of F_{ST}, beyond which power for STRUCTURE collapses and only the heterozygote-deficiency tests retain some, although low, power. The findings suggest that cases of cryptic sympatry may have passed unnoticed in population genetic screenings using number of loci typical of the pre-genomics era. Hence, cryptic sympatric populations may be more common than hitherto thought, and we urge more attention being diverted to their detection and characterization.

KEYWORDS
biodiversity monitoring, conservation management, genetic biodiversity, population genetic structure

1 | INTRODUCTION

Sympatric populations represent conspecific populations that coexist spatially during at least a part of their life cycle (Futuyama & Mayer, 1980; Mallet, Mayer, Nosil, & Feder, 2009). Such populations are of great interest in studies of ecological interaction and microevolutionary processes since their existence may represent the first steps of sympatric speciation processes (Maynard Smith, 1966; Via, 2001). They may reflect genetic adaptations to ecological niches and involve reproductive isolation occurring even in the absence of obvious migration barriers (Kawecki, 1996, 1997; Turelli, Barton, & Coyne, 2001).

From perspectives of management and conservation, sympatric populations are important to identify and monitor; they represent population diversity below the species level and such diversity has been documented to contribute to the portfolio effect in ecosystem stability (Schindler, Armstrong, & Reed, 2015; Schindler et al., 2010).

Further, genetic diversity is identified as the basis for all biological variation that should be protected and sustainably managed according to international agreements, such as the Convention on Biological Diversity (www.cbd.int).

Sympatric populations have been described in a wide range of taxa and ecosystems. Marine examples include sympatric killer whale populations specializing on different diets (Ford et al., 1998) and blue whales (Attard, Beheregaray, & Möller, 2016) and beluga whales on summer foraging migration (Hauser, Laidre, Ruydom, & Richard, 2014). In coastal waters, genetically differentiated, sympatric populations or "ecotypes" have been described for the littorina snail (Ravinet et al., 2016) and the Atlantic cod (Barth et al., 2017; Knutsen et al., 2018). Sympatric "clades" have been described for the blue swimming crab (Ren et al., 2018) and diatom plankton (De Decker et al., 2018). Many examples are known from freshwater environments, including smelts, whitefishes and salmonid fishes (reviewed by Taylor, 1999), and three-spined sticklebacks (Marques et al. (2016). Salmonid fishes seem particularly well represented in this regard (brown trout: Allendorf, Ryman, Stennek, & Ståhl, 1976; Andersson, Jansson et al., 2017; Ryman, Allendorf, & Ståhl, 1979; whitefish: Bernatchez & Dodson, 1990; Østbye, Næsje, Bernatchez, Sandlund, & Hindar, 2005). Examples from terrestrial ecosystems are sympatric populations of the meadow butterfly (Ford, 1975, p. 78–108), Asian tiger mosquitos (Guo et al., 2018) and ground beetles (Van Belleghem, De Wolf, & Hendrickx, 2016). A special case refers to sympatric "host races" of parasitic insects such as the apple maggot (Filchak, Roethele, & Feder, 2000) and pea aphid (Peccoud, Ollivier, Plantegenest, & Simon, 2009). Host-specific races are also known for the brood-parasitic common cuckoo, although it is unclear whether these "gentes" represent different populations or genetic polymorphism within populations (Fossøy et al., 2016). So-called "chromosome races" or "cytotypes" are known from small rodents that coexist in sympatry at least in zones of contact (house mouse: Corti & Rohlf, 2001; common screw: Orlov et al., 2012). In plants, there is a large literature on co-occurring populations that differ in ploidy (Schönswetter et al., 2007). A common pattern in most, but not all, of these instances is that members of the sympatric populations differ to some extent in visual characteristics and this appears to have been a key feature for detecting such populations.

Sympatric populations may be described as cryptic when causal inspection had not previously revealed clear morphological or behavioural differences between them (Bickford et al., 2007). In such situations, the detection of sympatric populations typically requires some form of genetic data. Whether cryptic or not, there is a problem of demarcating sympatric populations against sympatric, closely related, sister species. Researchers adhering to a strict interpretation of the biological species concept may classify all sympatric, reproductively isolated populations as full species. There are thus likely to be differences among taxa, ecosystems and field of research in the detection of cryptic biodiversity and how this diversity is recognized at the species level or below (Bickford et al., 2007; Struck et al., 2018). There is also the problem of defining sympatry: At what spatial and temporal scales should coexistence be defined? Sympatric populations may coexist in the same area only relatively briefly, for example during seasonal feeding migration (beluga whales: Hauser et al., 2014) or during their entire lifespan (brown trout: Ryman et al., 1979; Palmé, Laikre, & Ryman, 2013). Sympatry is more readily defined within a confined environment such as a lake or an island than in the open ocean or in open terrestrial landscapes, and this may add to differences among taxa and environments in recognition of the existence and occurrence of sympatry.

We hypothesize that cryptic sympatric populations may have gone largely undetected and therefore might be under-reported in the literature. First, sympatric populations in general may be perceived as somewhat of an exception under the dominating ecological view emphasizing niche specialization and competitive exclusion (Harding, 1960), possibly diverting attention away from a systematic search for them. Hence, phenotypically cryptic, sympatric populations may go unnoticed except as chance detection in genetic screenings for, for example genetic diversity assessment.

Second, the statistical power for detecting sympatric populations may be relatively low, at least in the absence of obvious phenotypic differences. Without observable phenotypic differences and using genetic data alone, the classical test for the presence of more than one population in a sample of individuals relies on the Wahlund effect, that is, a deficiency of heterozygotes relative to the Hardy–Weinberg expectation (Rousset & Raymond, 1995; Waples, 2015). Deviations from Hardy–Weinberg genotype proportions may have gone unnoticed due to low power of detection (Fairbairn & Roff, 1980). Indirect evidence that power of genetic methods has been weak is the observation that most cases of reported sympatry appear to be coupled to phenotypic differences (Taylor, 1999; this study). Individuals can then be grouped according to phenotype, and potential genetic differences between groups are investigated. This kind of comparison is frequently associated with higher statistical power than a general exploration of Hardy–Weinberg deviations (Palmé et al., 2013).

Third, as microsatellites became the marker of choice over allozymes, it early became clear that technical artefacts (allelic dropout: Taberlet et al., 1996; short allele dominance: Wattier, Engel, Saumitou-Laprade, & Valero, 1998; stutter bands: Miller & Yuan, 1997) and segregating null alleles (Chapuis & Estoup, 2007) all could lead to deficiencies of heterozygotes unrelated to any population mixture (Band & Ron, 1997). Concerns over such artefacts may have led researchers to dismiss also real heterozygote deficiencies and thereby overlook signals from population mixtures in their samples (Waples, 2015). More generally, studies reporting heterozygote deficiencies often fail to follow up on those observations with further investigations, and this lack of follow-up investigations leaves the possibility of population mixture unresolved (Castric, Bernatchez, Belkhir, & Bonhomme, 2002).

Finally, statistical tools beyond the Hardy–Weinberg test have traditionally been lacking for detecting mixtures of phenotypically cryptic populations occurring in sympatry. Mixture of genetically differentiated populations leads not only to non-random association of alleles within loci, but also among alleles at different loci (so-called "linkage" disequilibrium or LD; Makela & Richardson, 1977) and potentially more powerful methods that explore both effects to detect population mixture were not generally available until the turn

of the century (i.e., the STRUCTURE software: Pritchard, Stephens, & Donnelly, 2000). However, little is presently known about the statistical power of STRUCTURE relative to tests for heterozygote deficiency.

The purpose of the present paper is twofold. First, we review literature pertaining to sympatric populations. As pointed out above, there may be considerable differences among taxa and ecosystems with regard to how researchers recognize and interpret biological diversity. To maintain a level of consistency and uniformity, we therefore limit our review to freshwater salmonids, for which we have the most experience, sympatry is fairly easily defined and a relative rich literature exists. This review is pursued to summarize documentation of sympatric populations, particularly comparing the detection of cryptic, sympatric populations vs. non-cryptic detection, and further to find out whether commonly used genetic markers might have led to under-detection and hence under-reporting of sympatric populations. Second, we assess statistical power of detecting phenotypically cryptic populations from genetic data using computer simulations and focusing on realistic levels of genetic divergence, number of gene markers and sample sizes as revealed by the literature survey. The question addressed by these computer simulations is: what is the probability of detecting population admixture/structure from genotype data from a single sample or locality, that is, without additional information on habitat or phenotype differences?

2 | MATERIALS AND METHODS

2.1 | Literature survey

We carried out a literature survey on evidence for sympatric populations of salmonid fishes in freshwater environments using the Web of Science. We performed six topic searches using keyword combinations of "sympatric populations" AND either of the following "salmonid," "trout," "char," "charr," "whitefish" OR "salmon." The search included all years available in the database and was carried out in April 2018. In a next step, we examined the papers obtained for relevance with respect to our focus, that is, occurrence of sympatric populations in freshwater habitats. Further, we added nine papers that we knew of, but which did not appear in the searches. All in all, we included 80 studies in our survey. We classified the sympatric populations reported in these studies as *cryptic* if they were initially detected through genetics only, without prior identification of, or grouping based on, phenotypic, ecological or other divergence. The sympatric populations were classified as *non-cryptic* if the basis for detection was phenotypic differences and as *ambiguous* if they could not be classified as either cryptic or non-cryptic based on the information given in the studies.

2.2 | Computer simulations

Simulations employing an in-house computer program were used to assess statistical power of detecting a significant indication of population mixture in genetic data from a sample of individuals when no phenotypic or non-genetic cues to population membership exist. Simulated sample data sets were generated by random sampling from two interconnected populations in approximate migration-drift equilibrium, and statistical tests included Hardy–Weinberg tests equilibrium and tests derived from cluster analyses.

Each simulated population consisted of N = 1,000 diploid, sexually reproducing individuals with discrete (non-overlapping) generations. Populations were initiated with even sex ratios and with a number L of freely combining (i.e., unlinked) loci with a specified allele frequency profile. Various numbers of alleles (2 or 20) and loci (up to 100) were used to represent popular genetic marker types and numbers commonly used in past and present population screenings as identified in our literature review (Table 1). In particular, we consider a set of 10 loci with (initially) 20 alleles each and refer to this set as the "microsatellite panel" and a set of 100 di-allelic loci referred to as the "SNP panel." Smaller numbers of di-allelic loci were also simulated in order to represent allozyme-based studies. For the microsatellite panel, we incorporated mutations by randomly changing genes from their allelic state to one of the (19) other allelic states. We used a mutation rate of u = 0.0005, implying that one of the 2,000 genes in the population mutated per generation on average. The SNPs and allozymes were simulated without mutations. Simulations were initiated with even allele frequencies and were run for a sufficient number of generations (1,000) to thoroughly redistribute alleles within and among loci. Each generation after initiation (generation t = 0), N haploid gametes, including L loci plus the sex-determining locus, were drawn with replacement from males and from female parents, respectively, and merged into N diploid offspring which immediately replaced the parental generation. Thus, generations were discrete (non-overlapping) and population size was kept exactly constant, while the sex ratio varied randomly (i.e., binomially with a mean of 0.5 and a standard deviation of 0.0158). Migration was simulated by exchanging a fixed number (M) of diploid individuals between the two populations each generation, following reproduction and mutation. A range of levels of genetic divergence between populations (F_{ST}: from 0.00025 to 0.39) was generated by exchanging different numbers of migrants (M = 43, 23, 12, 5, 2.5, 1 or 0 per generation). Fractional numbers of migrants (e.g., 2.5) were accommodated by passing on the fractional part to the subsequent generation. Thus, in the case of M = 2.5, the actual number of migrants alternated between two and three in successive generations for an average of 2.5 per generation.

When sampling from the two populations, n_1 and n_2 diploid individuals were drawn from population 1 and 2, respectively, in generation t = 1,000 and both samples were pooled into a common file for statistical analyses. Different proportions of the two populations in samples were explored, from 1:1 (i.e., even representation) to 1:19 (highly skewed representation). The total sample size was set to cover the range over most empirical studies (Table 1), from 20 to 400 individuals combined ($n_1 + n_2$). When testing the case of no divergence (for assessment of alpha errors), that is F_{ST} = 0, a single, isolated population was simulated for 1,000 generations before the sample was drawn. Samples were drawn with replacement, in accordance with common—but typically not explicitly stated—assumptions of estimation procedures (Weir & Cockerham, 1984). The realized divergence (F_{ST}) between the two populations in the sample

TABLE 1 Summary information on sample size (loci and individuals) and F_{ST} found in the studies of the literature search (Table 2; Supporting Information Tables S1 and S2)

	Number of loci				Number of individuals				F_{ST}			
Marker type	Average	Median	Min	Max	Averag	Median	Min	Max	Average	Median	Min	Max
Allozymes	8.3	8	1	16	507	164	48	6,159	0.065	0.050	0.010	0.200
Microsatellites	8.7	6	5	22	139	81	22	636	0.106	0.062	0.007	0.381
SNPs	1,092.4	94	94	3,093	134	48	24	744	0.071	0.036	0.010	0.280

Note. Min: the smallest number of loci/individuals used and the smallest F_{ST} observed; Max: the largest number of loci/individuals employed and the largest F_{ST} observed.

was calculated from sample allele frequencies with GENEPOP (v. 4.2.1: Rousset, 2008, 2013) and compared to the expected theoretical value ($E[F_{ST}]$ calculated as β following Cockerham & Weir, 1987, p. 8513). In simulations without mutations, the theoretical values corresponding to the chosen parameters (M, N = 1,000 and t = 1,000) were $E[F_{ST}]$ = 0.0025, 0.0050, 0.0100, 0.0243, 0.0484, 0.1150 and 0.3912, for the different values of M, whereas in simulations that also included mutations (u = 0.0005), the corresponding expectations were slightly lower, at 0.0025, 0.0050, 0.0098, 0.0230, 0.0435, 0.0918 and 0.2589, respectively. All simulations and subsequent statistical analyses (below) were replicated 5,000 times.

2.3 | Statistical analyses of simulated data

Tests for heterozygote deficiency in the pooled samples (sample size = $n_1 + n_2$) utilized the sampled genotypes, anonymized with respect to population of origin by erasing the population identifiers from the input file prior to statistical analyses. The calculations were carried out with GENEPOP option 1 (Hardy–Weinberg exact test) suboption 4 (global tests for heterozygote deficiency), with default dememorization number (10,000), number of batches (20) and iterations per batch (5,000). Results were summarized as the proportion of the 5,000 replicate simulation runs that yielded a significant, at the 5% level, global test.

The pooled and anonymized samples were further analysed for population structure with the command line version of the STRUCTURE software (v. 2.3: Falush, Stephens, & Pritchard, 2003; Pritchard et al., 2000). The software was run with the default number of BURNINS (10,000) and NUMREPS (20,000) and, as per default, with the following settings activated (i.e., set to 1): FREQSCORR, COMPUTEPROBS, INFERALPHA or deactivated (set to 0): NOADMIX, USEPOPINFO, LOCPRIOR. For each simulation run, STRUCTURE was employed three times, with assumed number of populations (K) set to 1, 2 and 3, respectively. We chose 3 as an upper limit, partly to limit the computational burden (nearly 90% of CPU time was spent on the STRUCTURE analyses) and partly because few empirical investigators would consider a large number of populations in a single sample as a biologically realistic proposition. The posterior probability of K = 1 (i.e., the probability of the sample representing a single biological population) given the data were calculated from the reported Ln Prob(data|K) using Bayes' rule, as described in the manual (Pritchard, Xiaoquan, & Falush, 2010, section 5.1): Prob(K = 1|data) = exp[Prob(data|K = 1)]/(exp[Prob(data|K = 1)] + exp[Prob(data|K = 2)] + exp[Prob(data|K = 3)]). Results of simulation runs were summarized as the proportion of replicate runs that yielded a Prob(K = 1|data) less than 5% and interpreted as a significant (at the alpha = 5% level) detection of population mixing in the sample. In simulations involving a single population, that is with no true population mixing, the proportion of significant runs was interpreted as the alpha error of the test.

The discriminant analysis of the principal component method (DAPC: Jombart, Devillard, & Balloux, 2010), implemented in the R package ADEGENET (v.2.0.1: Jombart, 2008; Jombart & Ahmed, 2011), is also a potential tool for detecting the presence of individuals of different genetic origin within a sample. The *find.clusters* function calculates and reports the BIC (Bayesian information criterion) values for various number of clusters or populations (K) in the data. We did not find any application in the population genetics literature of using these BIC values to calculate posterior probabilities for the models, but the procedure is described in the general statistics literature on model selection (Burnham & Anderson, 2004, p. 275; Raftery, 1995) and is similar to that used for STRUCTURE. For testing the null hypothesis of a single population (K = 1) in the sample, we calculated Prob(K = 1|data) = exp(−1/2 delta BIC_K = 1)/sum (exp(−1/2 delta BIC_K = i)), where exp is the exponential function, BIC_K = i is the BIC value reported by the *find.clusters* function for K = i genetic clusters or populations, delta BIC_K = i is the difference between the BIC value for K = i and the lowest BIC value, and the summation is over i = 1…10. We calculated the power and alpha errors of this test as the proportions of replicate runs that yielded Prob(K = 1|data) < 0.05.

3 | RESULTS

3.1 | Literature survey

Review of the 80 papers identified in our literature survey revealed that for the case of salmonid fishes in freshwater habitats, sympatric populations have been reported in 136 cases in 135 localities in 17 countries, including at least 17 separate species (Table 2; Supporting Information Table S1). Arctic charr is the species with the largest number of reported sympatric cases with 39 localities where such existence has been documented. Sympatric populations have most commonly been found in freshwater lakes (108 cases), whereas river and creek habitats have been less commonly reported to harbour such populations (12 vs. 15 cases).

Based on the classification described in the methods section, we find that non-cryptic sympatric populations are much more commonly reported than cryptic ones. Non-cryptic populations have been documented in 98 localities involving 17 separate species (Table 2), whereas cryptic populations have only been documented in nine freshwater localities (seven lakes and two rivers) and for five species: Arctic charr, brown trout, lake trout, Atlantic salmon and Chinook salmon (Aykanat et al., 2015; Marin, Coon, Carson, Debes,

TABLE 2 Number of cases of sympatric populations in separate salmonid fishes in freshwater habitats classified as cryptic, non-cryptic and ambiguous (see text and Supporting Information Table S1 for details)

	Cryptic		Non-cryptic		Ambiguous		All sympatric
Species	Number of localities	Country (number of localities)	Number of localities	Country (number of localities)	Number of localities	Country (number of localities)	Total number of localities
Arctic charr (*Salvelinus alpinus*)	4[a]	Iceland (2), Scotland (2)	25	Canada (3), England (1), Iceland (5), Norway (3), Russia (10), Scotland (2), USA (1)	11	Iceland (4), Ireland (2), Scotland (5)	40
Arctic charr species complex (*Salvelinus spp.*)	0		10	Russia (8), USA (2)	2	USA (2)	12
Atlantic salmon (*Salmo salar*)	1	Finland/Norway (1)	2	Canada (2)	0		3
Brook charr (*Salvelinus fontinalis*)	0		1	Canada (1)	16	Canada (2), USA (14)	17
Brown trout (*Salmo trutta*)	2	Sweden (2)	3[a]	Ireland (1), Scotland (2)	0		5
Chinook salmon (*Oncorhynchus tshawytscha*)	1	USA (1)	1	Canada (1)	0		2
Cisco (*Coregonus albula*; *Coregonus fontanae*)	0		2	Germany (2)	0		2
European whitefish (*Coregonus lavaretus*)	0		14	Norway (13), Russia (1)	0		14
Lake trout (*Salvelinus namaycush*)	1	Canada (1)	1	Canada (1)	0		2
Lake whitefish (*Coregonus clupeaformis*)	0		9	Canada (6), USA (3)	0		9
Mediterranean/Fibreni trout (*Salmo cettii*; *Salmo fibreni*)	0		1	Italy (1)	0		1
Ohrid trout (*Salmo letnica typicus*; *Salmo letnica aestivalis*)	0		1	Albania/Macedonia (1)	0		1
Pygmy whitefish (*Prosopium coulterii*)	0		1	USA (1)	0		1
Rainbow smelt (*Osmerus mordax*)	0		1	Canada (1)	0		1
Rainbow/Steelhead trout (*Oncorhynchus mykiss*)	0		5	Canada (2), USA (3)	0		5
Sockeye/kokanee salmon (*Oncorhynchus nerka*)	0		11	Canada (121)	0		11
Whitefish (*Coregonus* spp.)	0		8	Switzerland (7) Switzerland/ Austria/ Germany (1)	0		8
Total (all species)	9[a]		98[a]		29		135

[a]Loch Awe in Scotland houses both two cryptic Arctic charr and two non-cryptic brown trout populations and is included both in the total number localities for both cryptic (9) and non-cryptic (98) populations. Thus, the total number of localities with sympatric populations identified in this study is 135.

& Fraser, 2016; Palmé et al., 2013; Ryman et al., 1979; Smith & Engle, 2011; Wilson et al., 2004; Table 1). We classify 29 cases concerning Arctic charr and brook charr as ambiguous. Most commonly, only two coexisting sympatric populations have been documented. A total of 23 cases with three or more coexisting populations have been found, and all these refer to non-cryptic populations (Supporting Information Table S1).

Difference in resource use has been reported in several cases of non-cryptic sympatry. In lake habitats, such differences include food niches (21 lakes), spawning time (3 lakes), spawning place (4), anadromous vs. resident strategy (2), both spawning time and place differences (3), and both spawning time and habitat differences (2). All non-cryptic sympatry in creeks is associated with anadromous vs. resident life history strategy (11 cases). In rivers, such differences are also found (three rivers) but here spawning time differences is another diverging factor (three rivers), whereas food niche separation has not been reported in creeks or rivers (Supporting Information Table S1).

In the cases of cryptic sympatry, clear life history strategy differences have only been observed in the case of Atlantic salmon in the Teno River (Aykanat et al., 2015). There are some indications of trophic divergence in sympatric charr in Lochs Maree and Stack (Adams, Wilson, & Ferguson, 2008) and extensive screening of the two cases of cryptic brown trout populations in tiny mountain lakes in Sweden found no trophic divergence but small growth and maturation differences as well as a tendency for a spacial separation at spawning (Andersson, Johansson, Sundbom, Ryman, & Laikre, 2017; Palmé et al., 2013; Ryman et al., 1979). Growth differences between cryptic, sympatric populations have been reported in a total of six cases (five lakes and one river).

Microsatellites and/or allozymes were the most frequently used markers for investigating genetic structure and had been employed in 40 vs. 21 studies, respectively. Typically, 1–16 loci were employed for allozymes and 5–22 for microsatellites. Four studies had used SNPs, five employed gene sequencing and several studies used combinations of different markers (Supporting Information Table S1). Studies identifying cryptic sympatric populations were based exclusively on heterozygote deficiency in one case (no heterozygotes observed; Ryman et al., 1979) and exclusively on STRUCTURE software in two cases (Aykanat et al., 2015; Marin et al., 2016). Two studies used a combination of heterozygote-deficiency tests and the STRUCTURE software (Palmé et al., 2013; Wilson et al., 2004), and one study applied an assignment software exclusively (ONCOR; Smith & Engle, 2011).

We wanted to find out if studies reporting cryptic vs. non-cryptic sympatric populations differed with respect to sample size, number of loci or degree of genetic divergence. For such a comparison, we selected studies reporting all the relevant quantities, that is number of fish, number of loci and significant F_{ST} (or equivalent). We limited our selection to studies using allozymes and/or microsatellites, since these were the most frequently applied markers. Of the 80 studies, 35 fulfilled these criteria and they represent 58 localities, seven with cryptic populations and 51 with non-cryptic ones (Supporting Information Tables S2). F_{ST} was consistently higher among cryptic populations as compared to non-cryptic ones using allozymes, microsatellites or a combination of both (Table 3). However, statistical significance was only obtained for allozymes using a *t* test (median test non-significant). Similarly, a larger number of individuals had been sampled in studies reporting cryptic populations based on allozymes or both markers as compared to studies reporting non-cryptic populations. However, this difference was only significant for the median test. The number of loci appointed were essentially the same (Table 3).

3.2 | Computer simulations

The overall impression from the computer simulations (Supporting Information Table S3) is that STRUCTURE was superior to DAPC and also more powerful than the heterozygote-deficiency test to detect

TABLE 3 Results from comparisons of genetic divergence (F_{ST}) and number of individuals and loci sampled between sympatric populations that were classified as cryptic or non-cryptic using information reported in the literature. Information from a total of 35 studies involving seven cases of cryptic populations and 59 cases of non-cryptic populations was used (Supporting Information Table S2). Probability values (*p*) below 0.05 are in bold. For measures with unequal variances, the Welch *t* test was used to estimate the *t*, *df* and *p*; these values are in italics. None of the significances were retained after Bonferroni correction

		T test					Median test	
Genetic marker type	Measure	Mean cryptic	Mean non-cryptic	*t*	*df*	*p*	χ^2	*p*
Allozymes + microsatellites	Total sample size	992.79	115.07	*1.02*	*6*	*0.348*	0.16	0.689
	Number of loci	9.29	8.76	0.33	64	0.742	0.38	0.535
	F_{ST}	0.13	0.10	0.81	64	0.422	4.00	0.046
Allozymes	Total sample size	3,212.00	108.00	*1.05*	*1*	*0.483*	4.44	0.035
	Number of loci	11.00	10.00	0.25	6	0.811	0.18	0.673
	F_{ST}	0.15	0.04	2.97	6	0.025	2.67	0.102
Microsatellites	Total sample ;size	105.10	115.87	−0.27	56	0.792	0.15	0.698
	Number of loci	8.60	8.62	−0.01	56	0.990	0.51	0.476
	F_{ST}	0.12	0.10	0.38	56	0.705	1.97	0.160

population mixture whenever the level of genetic divergence between the two populations was high ($F_{ST} \geq$ ~0.10). Power of all tests typically reached high levels—often unity—when the two populations were well differentiated and represented in even proportions in the sample (Figure 1). On the other hand, statistical power was very low when levels of genetic divergence were low and particularly so for DAPC and STRUCTURE. Our implementation of DAPC in these simulations was always inferior to the two other tests and often did not yield a meaningful result at all (i.e., a power of zero, unity or no estimate at all: cf. Figure 1; Supporting Information Table S3). Thus, the approach implemented in DAPC is not considered further in the present paper.

The two genetic marker panels that are the focus of the present simulations, the 10 microsatellite and 100 SNP panels, represent fairly similar amounts of genetic data. At the time of sampling in generation t = 1,000 approximately 11 out of an initial 20 alleles per microsatellite remained in the populations (with a mutation rate of u = 0.0005), resulting in a total of $10 \times (11 - 1) = 100$ more or less independent genes (i.e., observations) per sampled individual. At the same time, nearly all (>99%) SNPs were still polymorphic (two alleles each), and samples with this marker therefore had a similar number of $100 \times (2 - 1) = 100$ independent genes per individual sampled. Statistical power of the two marker panels was therefore, as expected, fairly similar (cf. Figure 1).

The power of STRUCTURE to detect population mixtures fell rather rapidly with declining levels of genetic divergence, and the rate of this decline was dependent on sample size (Figure 2) and number of loci (Figure 3). For a given sample size and genetic marker panel, a reduction in F_{ST} by a factor of 2 sometimes reduced power from very high to very low levels. For example, using the microsatellite panel and a sample size of n = 100 individuals (green line in Figure 2, upper right), power was 90% to detect an even mix of populations that diverged by F_{ST} = 0.0435 but power declined to only 17.5% when divergence was F_{ST} = 0.0250 (Supporting Information Table S3). For a similar sample size but using the SNP panel, the major drop in power (from 77.6% to just 2.3%) occurred at a somewhat lower F_{ST} level, between 0.025 and 0.010 (cf. Figure 2, lower panel and Supporting Information Table S3). These major drops in statistical power indicate the existence of certain "threshold" levels of divergence necessary for the STRUCTURE test to effectively detect population mixture. The effect of sample size on power for this test was proportional and predictable (Figure 2, right panels): Expressed in terms of this threshold F_{ST}, a reduction in sample size by a given factor led to a corresponding increase in the F_{ST} level that was required to maintain statistical power of detection. The effect on power of increasing or decreasing number of loci, assuming the same number of alleles per locus, was similar to the effect of increasing or decreasing sample size (Figure 3): An increase in number of loci typically resulted in a proportional reduction in the threshold F_{ST}.

For the heterozygote-deficiency test, power declined more slowly with reduced F_{ST} and eventually overtook as the more powerful test when F_{ST} fell below the threshold level for STRUCTURE (Figure 1). Nevertheless, power to detect heterozygote deficiencies at these low levels of divergence was poor in absolute terms, typically below 0.2, and implying that mixtures of weakly differentiated populations are likely to go unnoticed with either method, even if using large sample sizes (n = 400 individuals: Figure 2). In this parameter region with overall low power, there was generally a poor relationship between the realized $\hat{F}_{ST}$ in the individual computer runs and the outcome of the statistical tests in those runs (Figure 4, upper panels). This lack of correspondence between level of

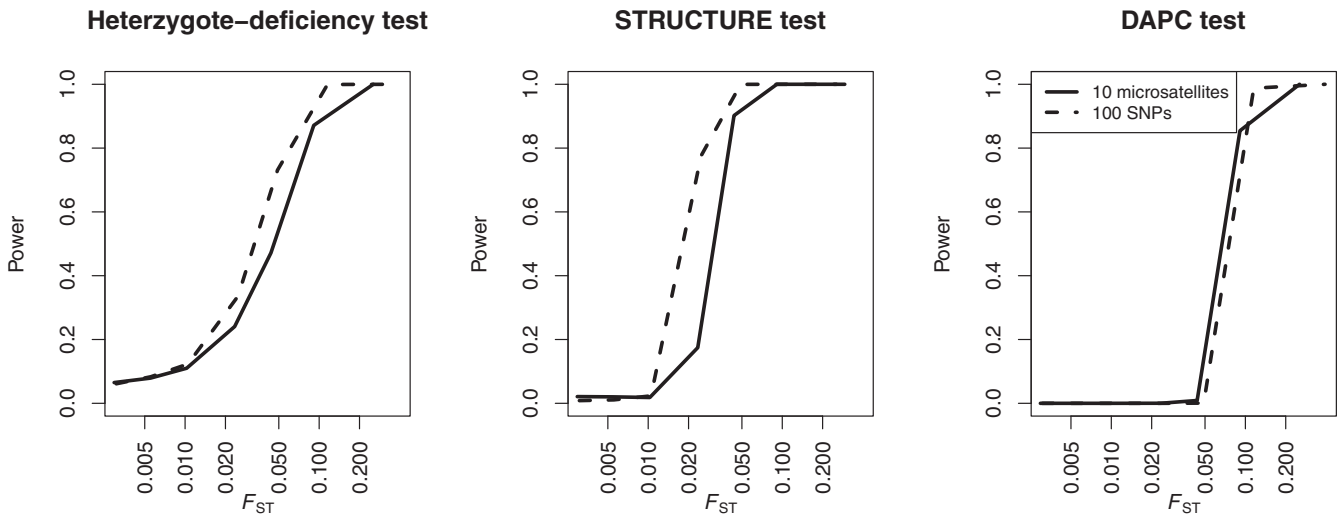

FIGURE 1 Simulated statistical power of detecting significant evidence for population mixture in a single sample genotyped for the microsatellite panel (10 loci with 20 alleles each: solid lines) and for the SNP panel (100 di-allelic loci: dashed lines). The total sample size was n = 100 individuals, drawn in equal proportions from the two populations. Three different tests were performed on the sample to detect population mixture, using GENEPOP (test for heterozygote deficiency; left), STRUCTURE (test for $K > 1$; middle) and DAPC (test for $K > 1$; right), and power was calculated as the proportion of significant (at the alpha = 0.05 level) simulation runs out of 5,000

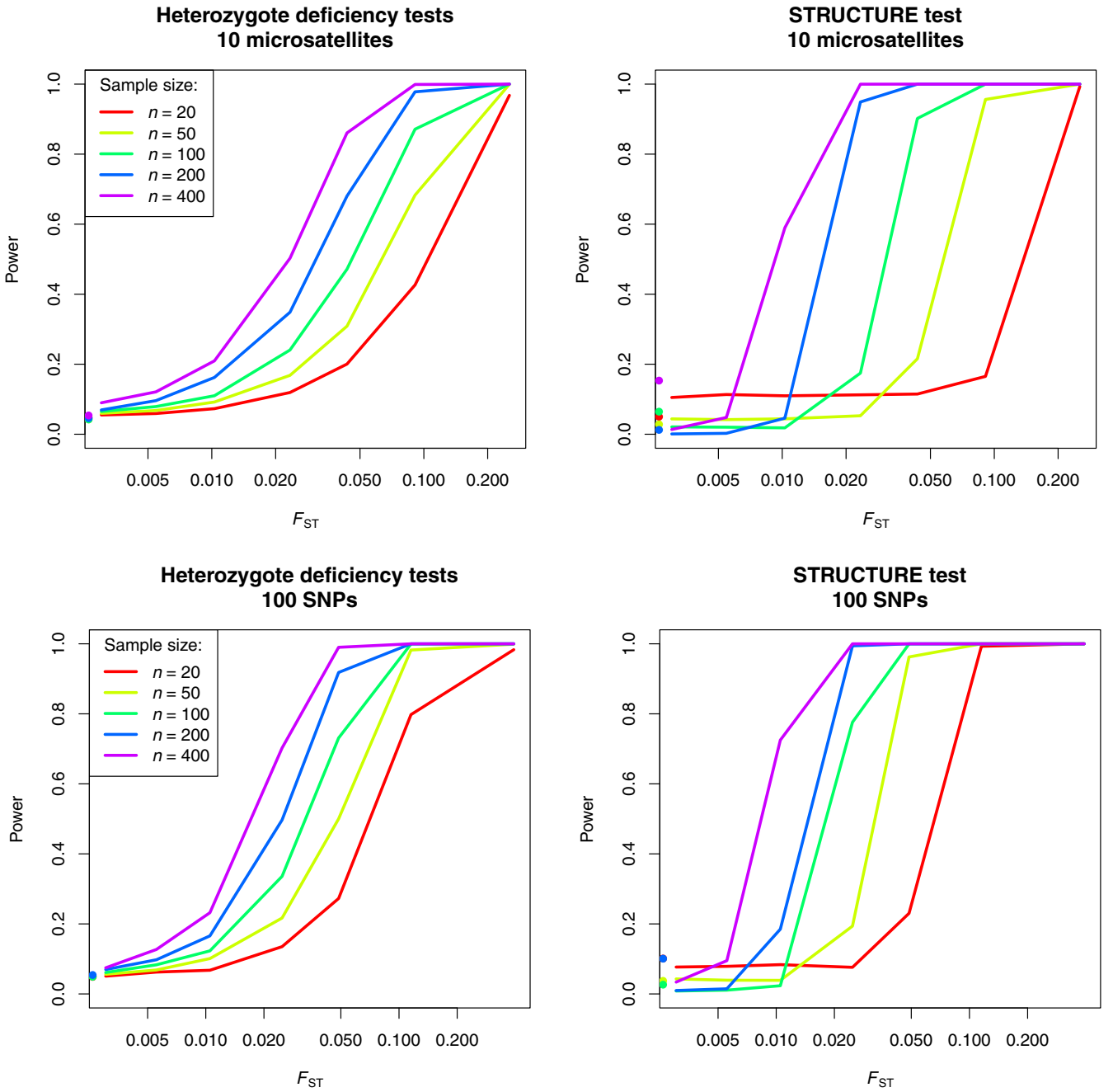

FIGURE 2 Power of detecting significant evidence for different size (*n* individuals), genotyped for the microsatellite panel (top) and the SNP panel (bottom) and tested with the GENEPOP heterozygote-deficiency test (left) and STRUCTURE test for $K > 1$ (right). Dots on the left margins indicate proportions of significant runs from a single, panmictic population and represent the alpha errors of the tests (note that some dots overlap)

divergence and test outcome likely reflected an increase in alpha errors (i.e., type I errors) relative to true rejections when true divergence was low. This sometimes resulted in contrasting outcomes of the two tests, with some runs being significant for the heterozygote-deficiency test and others for the STRUCTURE test and relatively few with both tests being significant. In the parameter region of higher overall power (Figure 4, bottom panels), such differences among tests rarely occurred and runs that were significant for the least powerful test (here, the heterozygote-deficiency test) were almost always significant also for the more powerful one (STRUCTURE).

With unequal sampling of the two populations, power of detecting mixtures declined with increasing skewness in population representation in the sample (Figure 5). While mild deviations of 30/70 from even proportions (50/50) only resulted in a minor effect on power, highly skewed representations of the two populations in the sample (10/90 and 5/95) typically limited power to a substantial

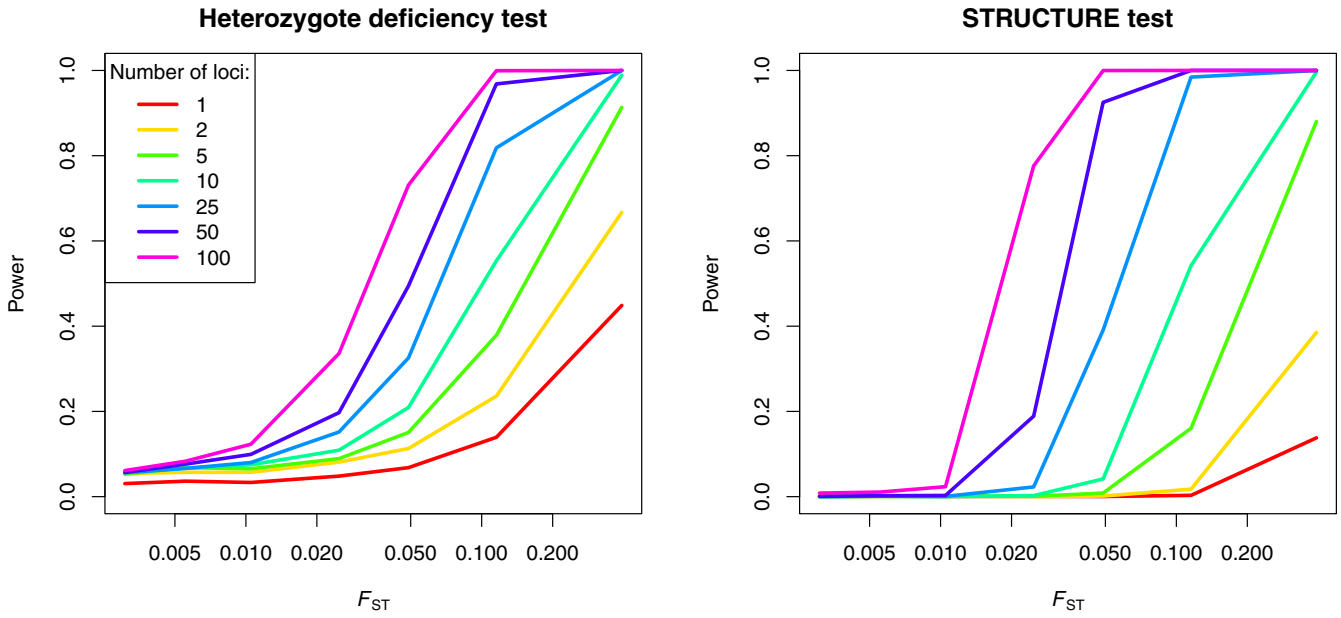

FIGURE 3 Power of detecting significant evidence for population mixture using various numbers of scored loci in a sample of fixed size (n = 100). All loci were di-allelic, without mutations, and tests refer to the GENEPOP heterozygote-deficiency test (left) and STRUCTURE test for $K > 1$ (right)

extent (Figure 5). STRUCTURE was somewhat more affected by uneven representation than was the heterozygosity-deficiency test but for highly divergent populations ($F_{ST} > 0.1$) power remained reasonable high (>0.5) for both methods also with highly skewed representation (5/95 proportions).

Alpha errors, that is, the proportion of simulation runs with a single, panmictic population only but which nevertheless resulted in a significant test outcome for mixture, were always close to the nominal alpha (5%) level for the heterozygote-deficiency test (cf. Figure 2), ranging between 0.042 and 0.061 over simulations representing a wide range of sample sizes (Supporting Information Table S3). The STRUCTURE test also tended to display alpha errors in the vicinity of the alpha level, but was more variable and ranged between 0.01 and 0.16 (Figure 2; Supporting Information Table S3).

4 | DISCUSSION

In our literature case study of sympatric populations using salmonid fishes in freshwater habitats as models, we found that the majority of reported cases—98 out of 136—refer to non-cryptic populations that were identified by differences in phenotypic and/or behavioural traits. Only nine of the 136 examples that we found refer to cryptic, sympatric populations, leaving the impression that such populations are rare. Moreover, we found that genetic divergence was on average higher between cryptic than between non-cryptic populations. This is contrary to expectation because phenotypically cryptic populations are commonly thought to be evolutionary young (see review and discussion by Fišer, Robinson, & Malard, 2018) and therefore less differentiated at neutral loci. Thus, the finding that cryptic populations instead tended to be more differentiated suggests that reported cases provide a biased view and represent situations where statistical power was high. Ecological divergence in sympatric populations appear to differ with respect to habitat but in the case of cryptic sympatry, obvious genetic differences are typically associated with only weak and unclear resource use divergence leaving the evolutionary mechanisms behind such structuring presently unclear.

Recent works that were not included in our literature review find refined food niche separation in three sympatric genetically divergent groups of brown trout in Loch Leidon, Scotland (Piggott et al., 2018), and genomic signals indicating selection between the non-cryptic life history forms of brown trout of Loch Maree, Scotland (Jacobs, Hughes, Robinson, Adams, & Elmer, 2018). Evidence for sympatric genetic divergence between non-cryptic Arctic charr populations was reported by Salisbury et al. (2018) in Ramah Lake in Labrador, Canada, and Guðbrandsson et al. (2018) found gene expression divergence during early development among non-cryptic populations of this species in Lake Thingvallavatn on Iceland .

We used computer simulations to evaluate the statistical power of methods that utilize genetic markers for detecting sympatric populations in a sample of individuals, without prior groupings. Among methods, the Hardy–Weinberg test represents the classical approach and different variants of this test have been developed. For the particular purpose of detecting Wahlund effects, the exact heterozygote-deficiency test (Rousset & Raymond, 1995) was used as this seems the most appropriate and has seen wide use in empirical studies (the original paper was cited >600 times at Web of Science by June 2018, but most papers using this method probably cite the GENEPOP papers, Raymond & Rousset, 1995; Rousset, 2008; instead, with a combined

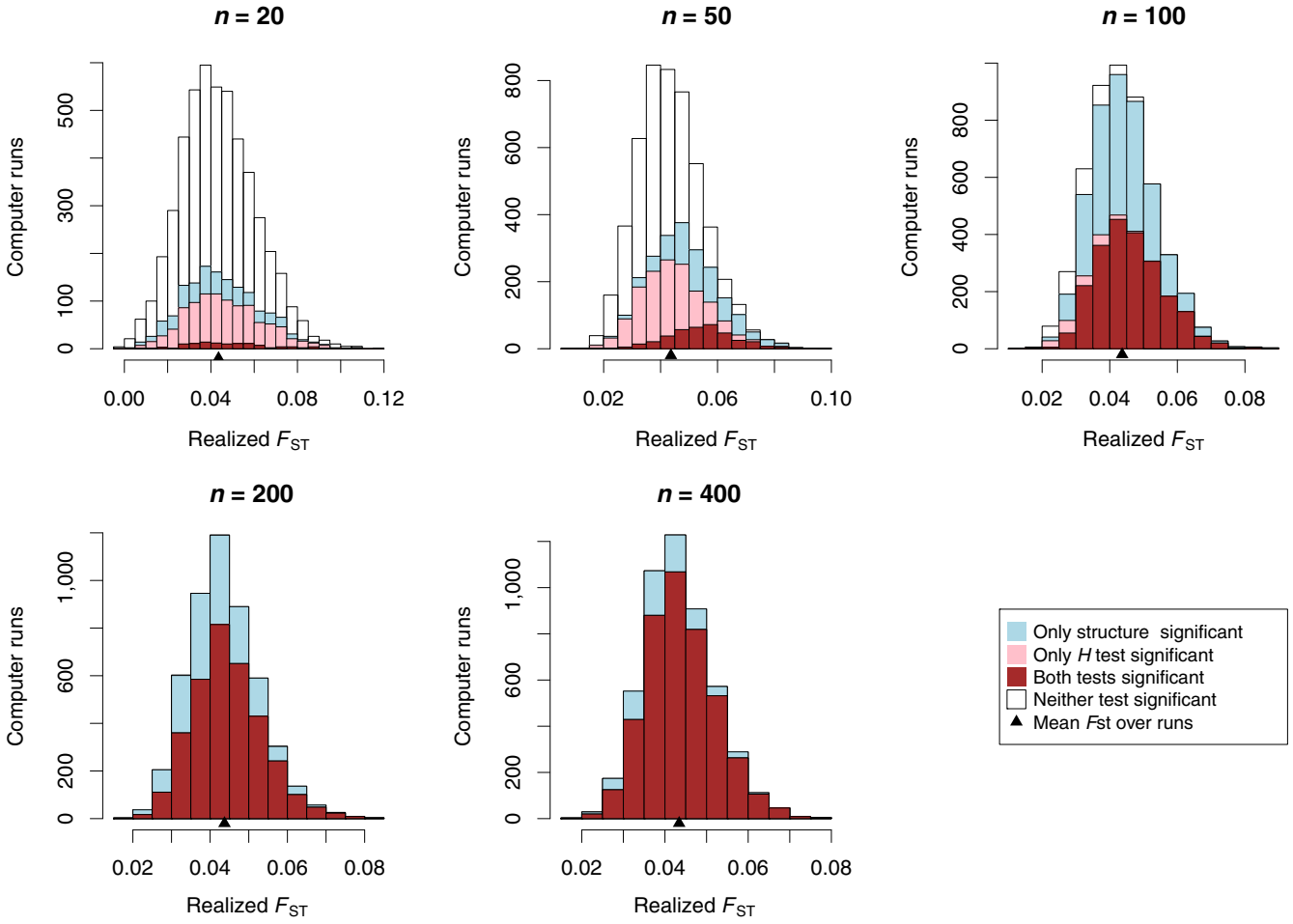

FIGURE 4 Distribution of $\hat{F}_{ST}$-estimates among 5,000 replicate computer runs of the microsatellite panel for various sample sizes (*n*). Simulations represent two populations (*N* = 1,000 each) exchanging *M* = 2.5 migrants per generation on average, with a predicted *E* [F_{ST}] = 0.0435 at time of sampling in generation *t* = 1,000. Populations were sampled in even proportions and coloured bars indicate successful detection (at the alpha = 5% level) of population mixture by either one (pink for heterozygote deficiency and light blue for STRUCTURE) or both (brown) statistical tests

>15,000 citations). Statistical power for Hardy–Weinberg tests in general and deficiency of heterozygotes in particular has been evaluated in the earlier literature for a variety of applications, including detection of cryptic population structure (Chakraborty & Zhong, 1994; Salanti, Amountza, Ntzani, & Ioannidis, 2005).

Methods that simultaneously utilize LD and heterozygote deficiencies have been developed, most notably in the STRUCTURE software (Pritchard et al., 2000), and been widely applied (>16,000 citations). Simulation studies characterizing statistical properties of STRUCTURE include Castric et al. (2002), Manel, Berthier, and Luikart (2002), Evanno, Regnaut, and Goudet (2005), Latch, Dharmarajan, Glaubitz, and Rhodes (2006), Patterson, Price, and Reich (2006), Waples and Gagiotti (2006), Anderson and Dunham (2008), Frantz, Cellina, Krier, Schley, and Burke (2009), Schwartz and McKelvey (2009), Jombart et al. (2010), Kalinowski (2011), Aurelle and Ledoux (2013), Neophytou (2014), Puechmaille (2016), Janes et al. (2017) and Wang (2017). These studies mainly addressed the problem of correctly estimating the number (*K*) of populations represented by a set of samples, and only Castric et al. (2002) specifically addressed the related but different problem of detecting a significant signal for $K > 1$. Nevertheless, these studies have yielded relevant information on the question at hand by demonstrating that STRUCTURE has problems with detecting populations with low genetic divergence (F_{ST} below about 0.01–0.05: Castric et al., 2002; Latch et al., 2006; Waples & Gagiotti, 2006). More specifically, Patterson et al. (2006) conjectured that *any* genetic clustering method, including STRUCTURE, will fail when $F_{ST} < 1/\sqrt{D}$, where *D* is the data size or the number of independent genes surveyed. It is not clear exactly when STRUCTURE "fails" in the present context of power analysis, but if we take a power of 10% as the point where there is no longer any obvious signal of population mixture, the "threshold" F_{ST} values that correspond to this power in the case of the SNP panel are about 0.027, 0.014, 0.011, 0.007 and 0.005 for sample sizes *n* = 20, 50, 100, 200 and 400, respectively (cf. Figure 2, bottom right). Because these are biallelic loci, there is only one independent allele per locus, so that with 100 loci $1/\sqrt{D} = 1/\sqrt{n \times 100 \times (2-1)}$ = 0.022, 0.014, 0.010, 0.007 and 0.005, in good agreement with the theoretical predictions. The agreement is less good for the microsatellite panel (with corresponding F_{ST} values of about 0.050, 0.030, 0.015,

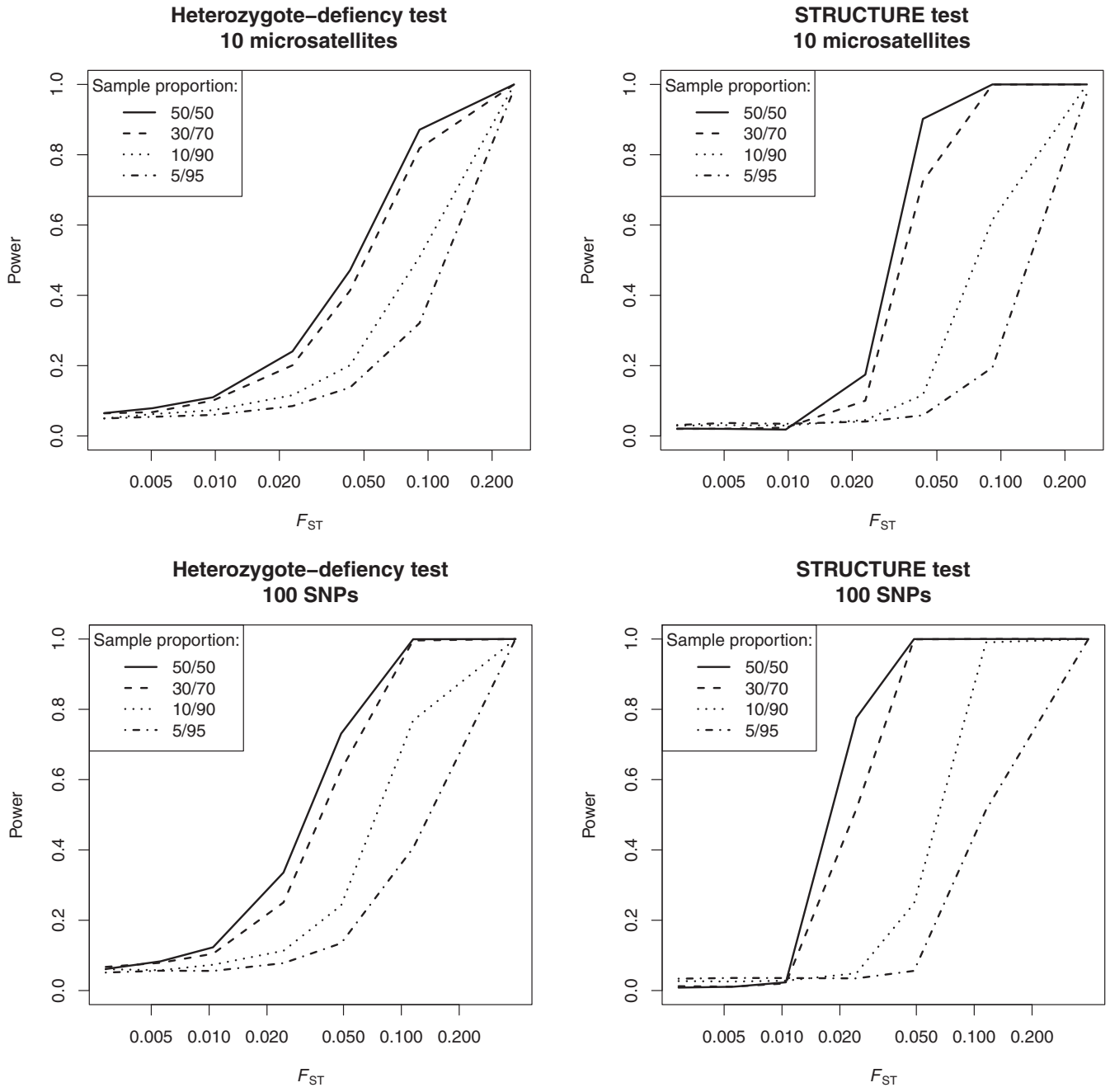

FIGURE 5 Power of detecting significant evidence for population mixture in a single sample of 100 individuals drawn from two populations in different proportions, from equal (50/50) to highly skewed (5/95), for different panels of genetic markers (top: microsatellite panel; bottom: SNP panel) and different statistical tests (left: heterozygote-deficiency test; right: STRUCTURE $K > 1$ test)

0.011 and 0.006, respectively: cf. Figure 2, top right), implying somewhat lower power for them, probably because alleles at the same locus are not entirely independent and that more alleles occurred in low frequencies as compared to the di-allelic SNPs. The expression is nevertheless useful also for microsatellites as a rough guideline for conditions where the statistical power of the STRUCTURE test becomes too low to be of practical use. However, this guideline refers to population mixtures in equal proportions and our simulation results show that power of STRUCTURE is reduced when populations are unequally represented in the sample (Figure 5, right), as also found earlier (Puechmaille, 2016; Wang, 2017). This reduction in power and subsequent increase in threshold F_{ST} appears to be directly related to the relative proportion of the two populations in the sample, or $r = n_1/n_2$ (where n_2 is the larger, so that $r \leq 1$). Using r, we may tentatively consider a modified expression for the threshold F_{ST} for STRUCTURE:

$$1/\sqrt{D} = 1/\sqrt{n \times r \times L \times (a-1)} \quad (1)$$

where n is the sample size, L the number of loci and a number of alleles per locus. With r = 5/95, 10/90, 30/70 and 50/50, as in our simulations (Figure 5), we then obtain for the 100 locus SNP panel

the following threshold F_{ST} values: 0.010, 0.015, 0.030 and 0.044, respectively. In comparing these numbers with simulation results, we extract F_{ST} values representing a power of 10% from Figure 5, bottom right, which turns out to be 0.011, 0.012, 0.029 and 0.053, respectively, in reasonable agreement with the calculations. These numbers imply that, with a skewed population representation, say 5/95, the level of genetic differentiation (F_{ST}) needs to be much higher, here 0.053/0.011 = 4.8 times higher, in order to achieve the same power of detection as with even population representation.

In comparison with results for STRUCTURE, power for the heterozygote-deficiency test decreased more gradually with decreasing F_{ST} (cf. Figures 1 and 2) and the concept of a lower "threshold" F_{ST} value is even less clear. Nevertheless, this test generally retained somewhat higher power than the STRUCTURE test at low levels of divergence, implying that a signal of sympatric mixture is slightly more likely to be picked up with the heterozygote-deficiency test. Apart from this slight advantage at low levels of divergence, the heterozygote-deficiency test displayed similar patterns of power depending on sample size, number of loci and population representation in the samples, as for STRUCTURE.

As discussed in the introduction, there are a number of circumstances that may have conspired against detecting sympatric populations in empirical genetic studies. Apart from situations where a significant test outcome is being dismissed or misinterpreted, our findings add insights into non-significant outcomes and aid in interpreting the current literature pertaining to the occurrence of cryptic, sympatric populations. Briefly, computer simulations verified that weakly divergent populations are likely to go undetected regardless of statistical test employed when number of loci and/or sample sizes are small. While we do not know what level of divergence may characterize real cryptic, sympatric populations, the literature review of non-cryptic populations showed that these diverged with a broad range of F_{ST} from 0.003 to 0.497, with an average of 0.095.

While recent advances in genomics have hugely expanded the number of loci that can be genotyped, most studies until recently were severely limited by available protein staining technology (for allozymes) or species-specific primers (for microsatellites). For studies employing microsatellites, numbers of loci in our literature survey ranged from 5 to 25 with a mean of 10, and for allozymes, the number of (polymorphic) loci was even lower. Combined with a moderate sample size, we conclude that most studies would not have been able to detect sympatric populations from genetic data alone. In the present genomics era increasing the number of loci substantially is no longer a problem, although sample sizes still tend to be moderately low.

Perhaps more problematic from a planning purpose is our finding that uneven population representation in the sample reduces power of detection substantially. Unless there is some unknown biological reason for sympatric populations to occur in even proportions in the sample area, simple combinatorics dictate that most samples will contain populations in uneven proportions and often highly so. As a case in point, both the sympatric brown trout populations in Lakes Bunnersjöarna and in Trollsvattnen occurred in very similar proportions, averaging 45% and 55% (Ryman et al., 1979) and 47% and 53% (Palmé et al., 2013), respectively. Although the alternative fixation of the LDH-1 alleles in Lake Bunnersjöarna makes statistical power in that particular case largely irrelevant, the high proportion of both types certainly brought attention to the phenomenon as not just a technical artefact with a few samples.

A complicating factor relating to detection of cryptic sympatry that we have not addressed here is that degree of divergence most likely differs in different regions of the genome. Such differences might explain the difficulty in detecting the two cryptic populations of brown trout in Lakes Trollsvattnen that we have reported and monitored over time (Andersson, Jansson et al., 2017; Andersson, Johansson et al., 2017; Jorde & Ryman, 1996; Palmé et al., 2013) with six microsatellites as compared to 14 allozyme loci. In fact, the degree of divergence between these population using allozymes is estimated as F_{ST} = 0.1, whereas when applying ~3,000 SNPs, we find a lower F_{ST} = 0.03 (Andersson, Jansson et al., 2017). Clearly, more research is needed into the issue of cryptic sympatry to understand the evolutionary background to their existence. From the perspective of conservation management, mapping the existence of this type of biodiversity over space and monitoring such existences over time is important.

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DATA ACCESSIBILITY

Results of the computer simulations and R-scripts for generating the figures described herein are available on Dryad https://doi.org/10.5061/dryad.689r9h2.

AUTHOR CONTRIBUTION

L.L., N.R., A.A. and P.E.J. designed the study; A.A. did the literature survey; and P.E.J. performed the computer simulations and wrote the first draft. All the authors contributed to the final version.

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REFERENCES

Adams, C. E., Wilson, A. J., & Ferguson, M. M. (2008). Parallel divergence of sympatric genetic and body size forms of Arctic charr, Salvelinus alpinus, from two Scottish lakes. *Biological Journal of the Linnean*

Society, *95*, 748–757. https://doi.org/10.1111/j.1095-8312.2008.01066.x

Allendorf, F., Ryman, N., Stennek, A., & Ståhl, G. (1976). Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): Evidence of distinct sympatric populations. *Hereditas*, *83*, 73–82.

Anderson, E. C., & Dunham, K. K. (2008). The influence of family groups on inferences made with the program Structure. *Molecular Ecology Resources*, *8*, 1219–1229. https://doi.org/10.1111/j.1755-0998.2008.02355.x

Andersson, A., Jansson, E., Wennesrström, L., Chiriboga, F., Arnyasi, M., Kent, M. P., … Laikre, L. (2017). Complex genetic diversity patterns of cryptic, sympatric brown trout (*Salmo trutta*) populations in tiny mountain lakes. *Conservation Genetics*, *18*, 1213–1227. https://doi.org/10.1007/s10592-017-0972-4

Andersson, A., Johansson, F., Sundbom, M., Ryman, N., & Laikre, L. (2017). Lack of trophic polymorphism despite substantial genetic differentiation in sympatric brown trout (*Salmo trutta*) populations. *Ecology of Freshwater Fish*, *26*, 643–652. https://doi.org/10.1111/eff.12308

Attard, C. R. M., Beheregaray, L. B., & Möller, L. M. (2016). Towards population-level conservation in the critically endangered Antarctic blue whale: The number and distribution of their populations. *Scientific Reports*, *6*, 22291. https://doi.org/10.1038/srep22291

Aurelle, D., & Ledoux, J.-B. (2013). Interplay between isolation by distance and genetic clusters in the red coral *Corallium rubrum*: Insights from simulated and empirical data. *Conservation Genetics*, *14*, 705–716. https://doi.org/10.1007/s10592-013-0464-0

Aykanat, T., Johnston, S. E., Orell, P., Niemelä, E., Erkinaro, J., & Primmer, C. R. (2015). Low but significant genetic differentiation underlies meaningful phenotypic divergence in a large Atlantic salmon population. *Molecular Ecology*, *24*, 5158–5174. https://doi.org/10.1111/mec.13383

Band, M., & Ron, M. (1997). Heterozygote deficiency caused by a null allele at the bovine ARO23 microsatellite. *Animal Biotechnology*, *8*, 187–190. https://doi.org/10.1080/10495399709525881

Barth, J. M. I., Berg, P. R., Jonsson, P. R., Bonanomi, S., Corell, H., Hemmer-Hansen, J., … André, C. (2017). Genome architecture enables local adaptation of Atlantic cod despite high connectivity. *Molecular Ecology*, *26*, 4452–4466. https://doi.org/10.1111/mec.14207

Bernatchez, L., & Dodson, J. J. (1990). Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) as revealed by mitochondrial DNA restriction analysis. *Evolution*, *44*, 1263–1271.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., … Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, *22*, 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods & Research*, *33*, 261–304. https://doi.org/10.1177/0049124104268644

Castric, V., Bernatchez, L., Belkhir, K., & Bonhomme, F. (2002). Heterozygote deficiencies in small lacustrine populations of brook charr Salvelinus Fontinalis Mitchill (Pisces, Salmonidae): A test of alternative hypotheses. *Heredity*, *89*, 27–35. https://doi.org/10.1038/sj.hdy.6800089

Chakraborty, R., & Zhong, Y. (1994). Statistical power of an exact test of Hardy-Weinberg proportions of genotypic data at a multiallelic locus. *Human Heredity*, *44*, 1–9. https://doi.org/10.1159/000154181

Chapuis, M.-P., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, *24*, 621–631. https://doi.org/10.1093/molbev/msl191

Cockerham, C. C., & Weir, B. S. (1987). Correlations, descent measures: Drift with migration and mutation. *Proceedings of the National Academy of Sciences of the United States of America*, *84*, 8512–8514. https://doi.org/10.1073/pnas.84.23.8512

Corti, M., & Rohlf, F. J. (2001). Chromosomal speciation and phenotypic evolution in the house mouse. *Biological Journal of the Linnean Society*, *73*, 99–112. https://doi.org/10.1111/j.1095-8312.2001.tb01349.x

De Decker, S., Vanormelingen, P., Pinseel, E., Sefbom, J., Audoor, S., Sabbe, K., & Vyverman, W. (2018). Incomplete reproductive isolation between genetically distinct sympatric clades of the pennate model diatom *Seminavis robusta*. *Protist*, *169*, 569–583. https://doi.org/10.1016/j.protis.2018.05.003

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fairbairn, D. J., & Roff, D. A. (1980). Testing genetic models of isozyme variability without breeding data: Can we depend on the χ^2? *Canadian Journal of Fisheries and Aquatic Sciences*, *37*, 1149–1159. https://doi.org/10.1139/f80-147

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*, 1567–1587.

Filchak, K. E., Roethele, J. B., & Feder, J. L. (2000). Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature*, *407*, 739–742. https://doi.org/10.1038/35037578

Fišer, C., Robinson, C. T., & Malard, F. (2018). Cryptic species as a window into the paradigm shift of the species concept. *Molecular Ecology*, *27*, 613–635.

Ford, E. B. (1975). *Ecological genetics* (4th ed., p. 442). London, UK: Chapman & Hall.

Ford, J. K. B., Ellis, G. M., Barrett-Lennard, L. G., Morton, A. B., Palm, R. S., & Balcomb, K. C. III (1998). Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent waters. *Canadian Journal of Zoology*, *76*, 1456–1471. https://doi.org/10.1139/z98-089

Fossøy, F., Sorenson, M. D., Liang, W., Ekrem, T., Moksnes, A., Møller, A. P., … Stokke, B. G. (2016). Ancient origin and maternal inheritance of blue cuckoo eggs. *Nature communications*, *7*, 10272. https://doi.org/10.1038/ncomms10272

Frantz, A. C., Cellina, S., Krier, A., Schley, L., & Burke, T. (2009). Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: Clusters or isolation by distance? *Journal of Applied Ecology*, *46*, 493–505. https://doi.org/10.1111/j.1365-2664.2008.01606.x

Futuyama, D. J., & Mayer, G. C. (1980). Non-allopatric speciation in animals. *Systematic Zoology*, *29*, 254–271. https://doi.org/10.2307/2412661

Guðbrandsson, J., Franzdóttir, S. R., Kristjánsson, B. K., Ahi, E. P., Maier, V. H., Kapralova, K. H., … Pálsson, A. (2018). Differential gene expression during early development in recently evolved and sympatric Arctic charr morphs. *PeerJ*, *6*, e4345. https://doi.org/10.7717/peerj.4345

Guo, Y., Song, Z., Luo, L., Wang, Q., Zhou, G., Yang, D., … Zheng, X. (2018). Molecular evidence for new sympatric cryptic species of *Aedes albopictus* (Diptera: Culicidae) in China: A new threat from *Aedes albopictus* subgroup? *Parasites & Vectors*, *11*, 228. https://doi.org/10.1186/s13071-018-2814-8

Harding, G. (1960). The competitive exclusion principle. *Science*, *131*, 1292–1297. https://doi.org/10.1126/science.131.3409.1292

Hauser, D. D. W., Laidre, K. L., Ruydom, R. S., & Richard, P. R. (2014). Population-specific home ranges and migration timing of Pacific Arctic beluga whales (*Delphinapterus leucas*). *Polar Biology*, *37*, 1171–1183. https://doi.org/10.1007/s00300-014-1510-1

Jacobs, A., Hughes, M. R., Robinson, P. C., Adams, C. E., & Elmer, K. R. (2018). The genetic architecture underlying the evolution of a rare piscivorous life history form in brown trout after secondary contact and strong introgression. *Genes*, *9*, 280. https://doi.org/10.3390/genes9060280

Janes, J. K., Miller, J. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., Cullingham, C. I., & Andrew, R. L. (2017). The K=2 conundrum. *Molecular Ecology*, *26*, 3594–3602. https://doi.org/10.1111/mec.14187

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Jorde, P. E., & Ryman, N. (1996). Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics*, *143*, 1369–1381.

Kalinowski, S. T. (2011). The computer program STRUCTURE does not reliably identify the main genetic clusters within species: Simulations and implications for human population structure. *Heredity*, *106*, 625–632. https://doi.org/10.1038/hdy.2010.95

Kawecki, T. J. (1996). Sympatric speciation driven by beneficial mutations. *Proceedings of the Royal Society B: Biological Sciences*, *263*, 1515–1520. https://doi.org/10.1098/rspb.1996.0221

Kawecki, T. J. (1997). Sympatric speciation via habitat specialization driven by deleterious mutations. *Evolution*, *51*, 1751–1763. https://doi.org/10.1111/j.1558-5646.1997.tb05099.x

Knutsen, H., Jorde, P. E., Hutchings, G. A., Hemmer-Hansen, J., Grønkjær, P., Mose Jørgensen, K.-E., … Olsen, E. M. (2018). Stable coexistence of genetically divergent Atlantic cod ecotypes at multiple spatial scales. *Evolutionary Applications*, 1–13 . https://doi.org/10.1111/eva.12640

Latch, E. K., Dharmarajan, G., Glaubitz, J. C., & Rhodes, O. E. Jr (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, *7*, 295–302. https://doi.org/10.1007/s10592-005-9098-1

Makela, M. E., & Richardson, R. H. (1977). The detection of sympatric sibling species using genetic correlation analysis. I. Two loci, two gamodemes. *Genetics*, *86*, 665–678.

Mallet, J., Mayer, A., Nosil, P., & Feder, J. L. (2009). Space, sympatry and speciation. *Journal of Evolutionary Biology*, *22*, 2332–2341. https://doi.org/10.1111/j.1420-9101.2009.01816.x

Manel, S., Berthier, P., & Luikart, G. (2002). Detecting wildlife poaching: Identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology*, *16*, 650–659. https://doi.org/10.1046/j.1523-1739.2002.00576.x

Marin, K., Coon, A., Carson, R., Debes, P. V., & Fraser, D. J. (2016). Striking phenotypic variation yet low genetic differentiation in sympatric lake trout (*Salvelinus namaycush*). *PLoS One*, *11*, e0162325. https://doi.org/10.1371/journal.pone.0162325

Marques, D. A., Lucek, K., Meier, J. I., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2016). Genomics of rapid incipient speciation in sympatric threespine stickleback. *PLoS Genetics*, *12*(2), e1005887.

Maynard Smith, J. (1966). Sympatric speciation. *American Naturalist*, *100*, 637–650. https://doi.org/10.1086/282457

Miller, M. J., & Yuan, B.-Z. (1997). Semiautomated resolution of overlapping stutter patterns in genomic microsatellite analysis. *Analytical Biochemistry*, *251*, 50–56. https://doi.org/10.1006/abio.1997.2234

Neophytou, C. (2014). Bayesian clustering analyses for genetic assignment and study of hybridization in oaks: Effects of asymmetric phylogenies and asymmetric sampling schemes. *Tree Genetics & Genomes*, *10*, 273–285. https://doi.org/10.1007/s11295-013-0680-2

Orlov, V. N., Borisov, Y. M., Cherepanova, E. V., Grigor'eva, O. O., Shestak, A. G., & Sycheva, V. B. (2012). Narrow hybrid zone between Moscow and Western Dvina chromosomal races and specific features of population isolation in common shrew *Sorex araneus* (Mammalia). *Russian Journal of Genetics*, *48*, 70–78. https://doi.org/10.1134/S1022795412010152

Østbye, K., Næsje, T. F., Bernatchez, L., Sandlund, O. T., & Hindar, K. (2005). Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway. *Journal of Evolutionary Biology*, *18*, 683–702.

Palmé, A., Laikre, L., & Ryman, N. (2013). Monitoring reveals two genetically distinct brown trout populations remaining in stable sympatry over 20 years in tiny mountain lakes. *Conservation Genetics*, *14*, 795–808. https://doi.org/10.1007/s10592-013-0475-x

Patterson, N., Price, A. L., & Reich, D. (2006). Population structure and eigenanalysis. *PLoS Genetics*, *2*, 2074–2093.

Peccoud, J., Ollivier, A., Plantegenest, M., & Simon, J.-C. (2009). A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *PNAS*, *106*, 7495–7500. https://doi.org/10.1073/pnas.0811117106

Piggott, C. V. H., Verspoor, E., Greer, R., Hooker, O., Newton, J., & Adams, C. E. (2018). Phenotypic and resource use partitioning amongst sympatric, lacustrine brown trout, *Salmo trutta*. *Biological Journal of the Linnean Society*, *124*, 200–212. https://doi.org/10.1093/biolinnean/bly032

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Pritchard, J. K., Xiaoquan, W., & Falush, D. (2010). *Documentation for structure software: Version 2.3*. Retrieved from: https://web.stanford.edu/group/pritchardlab/structure_software/release_versions/v2.3.4/structure_doc.pdf

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*, 608–627. https://doi.org/10.1111/1755-0998.12512

Raftery, A. E. (1995). Bayesian model selection in social research. *Sociological Methodology*, *25*, 11–163.

Ravinet, M., Westram, A., Johannesson, K., Butlin, R., André, C., & Panova, M. (2016). Shared and nonshared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale. *Molecular Ecology*, *25*, 287–305. https://doi.org/10.1111/mec.13332

Raymond, M., & Rousset, F. (1995). Genepop (version 1.2): Population genetics software for exact tests and ecumenism. *The Journal of Heredity*, *86*, 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Ren, G., Miao, G., Ma, C., Lu, J., Yang, X., & Ma, H. (2018). Genetic structure and historical demography of the blue swimming crab (*Portunus pelagicus*) from southeastern sea of China based on mitochondrial COI gene. *Mitochondrial DNA Part A*, *29*, 192–198. https://doi.org/10.1080/24701394.2016.1261855

Rousset, F. (2008). Genepop'007: A complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rousset, F. (2013). *Genetic structure and selection in subdivided populations*. Princeton, NJ: Princeton University Press.

Rousset, F., & Raymond, M. (1995). Testing heterozygote excess and deficiency. *Genetics*, *140*, 1413–1419.

Ryman, N., Allendorf, F. W., & Ståhl, G. (1979). Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). *Genetics*, *92*, 247–262.

Salanti, G., Amountza, G., Ntzani, E. E., & Ioannidis, J. P. A. (2005). Hardy-Weinberg equilibrium in genetic association studies: An empirical evaluation of reporting, deviations, and power. *European Journal of Human Genetics*, *13*, 840–848. https://doi.org/10.1038/sj.ejhg.5201410

Salisbury, S. J., Booker, C., McCracken, G. R., Knight, T., Keefe, D., Perry, R., & Ruzzante, D. E. (2018). Genetic divergence among and within Arctic char (*Salvelinus alpinus*) populations inhabiting landlocked and sea-accessible sites in Labrador, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*, 1256–1269. https://doi.org/10.1139/cjfas-2017-0163

Schindler, D. E., Armstrong, J. B., & Reed, T. E. (2015). The portfolio concept in ecology and evolution. *Frontiers in Ecology and Environment*, *13*, 257–263. https://doi.org/10.1890/140275

Schindler, D. E., Hilborn, R., Chasco, B., Boatright, C. P., Quinn, T. P., Rogers, L. A., & Webster, M. S. (2010). Population diversity and the portfolio effect in an exploited species. *Nature*, *465*, 609–612. https://doi.org/10.1038/nature09060

Schönswetter, P., Lachmayer, M., Lettner, C., Prehsler, P., Rechnitzer, S., Reich, D. S., … Suda, J. (2007). Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research*, *120*, 721–725. https://doi.org/10.1007/s10265-007-0108-x

Schwartz, M. K., & McKelvey, K. S. (2009). Why sampling scheme matters: The effect of sampling scheme on landscape genetic results. *Conservation Genetics*, *10*, 441–452. https://doi.org/10.1007/s10592-008-9622-1

Smith, C. T., & Engle, R. (2011). Persistent reproductive isolation between sympatric lineages of fall Chinook salmon in White Salmon River, Washington. *Transactions of the American Fisheries Society*, *140*, 699–715. https://doi.org/10.1080/00028487.2011.584490

Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., … Dimitrov, D. (2018). Finding evolutionary processes hidden in cryptic species. *Trends in Evology and Evolution*, *33*, 153–163. https://doi.org/10.1016/j.tree.2017.11.007

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., … Bouvet, J. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, *26*, 3189–3194. https://doi.org/10.1093/nar/24.16.3189

Taylor, E. B. (1999). Species pairs of north temperate freshwater fishes: Evolution, taxonomy, and conservation. *Reviews in Fish Biology and Fisheries*, *9*, 299–324. https://doi.org/10.1023/A:1008955229420

Turelli, M., Barton, N. H., & Coyne, J. A. (2001). Theory and speciation. *Trends in Ecology and Evolution*, *16*, 330–343. https://doi.org/10.1016/S0169-5347(01)02177-2

Van Belleghem, S. M., De Wolf, K., & Hendrickx, F. (2016). Behavioral adaptations imply a direct link between ecological specialization and reproductive isolation in a sympatrically diverging ground beetle. *Evolution*, *70*, 1904–1912. https://doi.org/10.1111/evo.12998

Via, S. (2001). Sympatric speciation in animals: The ugly duckling grows up. *Trends in Ecology and Evolution*, *16*, 381–390. https://doi.org/10.1016/S0169-5347(01)02188-7

Wang, J. (2017). The computer program STRUCTURE for assigning individuals to populations: Easy to use but easier to misuse. *Molecular Ecology Resources*, *17*, 981–990. https://doi.org/10.1111/1755-0998.12650

Waples, R. S. (2015). Testing for Hardy-Weinberg proportions: Have we lost the plot? *Journal of Heredity*, *106*, 1–19. https://doi.org/10.1093/jhered/esu062

Waples, R. S., & Gagiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, *15*, 1419–1439. https://doi.org/10.1111/j.1365-294X.2006.02890.x

Wattier, R., Engel, C. R., Saumitou-Laprade, P., & Valero, M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: Experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, *7*, 1569–1573. https://doi.org/10.1046/j.1365-294x.1998.00477.x

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.

Wilson, A. J., Gíslason, D., Skúlason, S., Snorrason, S. S., Adams, C. E., Alexander, G., … Ferguson, M. M. (2004). Population genetic structure of Arctic Charr, *Salvelinus alpinus* from northwest Europe on large and small spatial scales. *Molecular Ecology*, *13*, 1129–1142. https://doi.org/10.1111/j.1365-294X.2004.02149.x

SUPPORTING INFORMATION

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