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Intraspecific diversity in Arctic charr, *Salvelinus alpinus*, in Iceland: I. Detection using mixture models

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ABSTRACT

Background: Finite-mixture models allow sub-populations to be described by separate parameter sets, but do not require the members of sub-populations to be identified. Although the accessibility of these methods has greatly increased in recent years, they are rarely used in studies of ecology and evolution, despite their ability to address basic and common questions regarding the detection of biological diversity.

Questions: Do mixture models uncover previously unknown polymorphism of Arctic charr (*Salvelinus alpinus*) within populations across Iceland? How many groups can be defined based on morphology and growth curves? How differentiated are these groups, and do these groups correspond with other differences, e.g. in life history and habitat use?

Data incorporated: Length-at-age, maturity, diet, and morphology data on Arctic charr from 50 lakes sampled in the Ecological Survey of Icelandic Lakes.

Analysis methods: We used mixture models to detect polymorphism as the presence of (1) multiple von Bertalanffy growth curves and (2) multimodality in multivariate morphology. We also analysed whether timing of maturity was related to growth and whether sexual dimorphism confounded the results. Dietary patterns were used to confirm a relationship between polymorphism and resource use. Metrics were defined to indicate the relative degree of differentiation.

Conclusions: Polymorphism occurs in more lakes than previously documented. In many cases, polymorphism in growth curves or morphology was mirrored by differences in diet. Mixture models are useful tools for identifying multimodal diversity below the species level (e.g. resource polymorphism). These results yield an important first step in understanding how the functional role of a species can vary within and among localities and how this may relate to processes of divergence.

Keywords: bimodal distribution, divergent selection, ecological speciation, individual heterogeneity, life history, maturity, mixture model, resource polymorphism, salmonid, Von Bertalanffy.

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INTRODUCTION

Finite-mixture models are general probabilistic models that allow sub-populations to be described by separate sets of parameters, but do not require that the members of the sub-population are identified (McLachlan and Peel, 2000). As McLachlan and Peel (2000) noted, flexibility in representing a wide variety of distributional shapes has led to the incorporation of mixture modelling into many scientific fields. Although statistical and computational advances have greatly improved the accessibility and diversity of methods based on mixture models (McLachlan and Peel, 2000), they are rarely used to address basic questions common in ecology and evolutionary studies, such as, 'How many groups comprise my population?' and 'Which individuals belong to which group?' Not only are the answers to these questions prerequisites for the assumption of homogeneity in statistical inference, they are also fundamental for interpreting ecological or evolutionary data; the biological unit under examination must first be identified.

Ironically, one of the first applications of mixture models was to resolve the classic questions outlined above: it was used to show the presence of two sub-species in a sampled crab population (as described by McLachlan and Peel, 2000). In addition, there has been increasing acknowledgment that intraspecific diversity may affect parameter estimation in population biology and resource management, for example as 'individual heterogeneity', a term that has been used to account for any biological deviations from the assumption of independence and identical distribution. Examples of individual heterogeneity include spatial variation in growing conditions, survival or capture probabilities, or other causes of individual variability (Cubaynes *et al.*, 2012). Therefore, mixture models have recently been developed as an important component of more complex models that allow population components to be reflected by separate parameter estimations. Examples include capture–recapture modelling (Royle, 2004), classification routines (Webb *et al.*, 2007), and analyses of stock mixtures (e.g. Podlaski, 2010; Smith and Campana, 2010; Thorson *et al.*, 2011). However, mixture models have rarely been used for the more basic goals of identifying and characterizing intraspecific or other levels of diversity (i.e. as opposed to simply gaining a better model fit). Given that biological diversity is valued not only for aesthetics (Jepson and Canney, 2003) but also as an integral component of ecosystem functions (Hilborn *et al.*, 2003; Cardinale *et al.*, 2006), the infrequent use of more basic mixture models is surprising. There may be various reasons for this, but we believe it is simply due to a lack of familiarity by ecologists and evolutionary biologists.

The goal of this study was to demonstrate how simple mixture models can be used to fill a gap in the statistical tool kit used by ecologists and evolutionary biologists. The emphasis placed today on analysis of variance (ANOVA) and generalized linear models in the standard training of ecologists and evolutionary biologists may unduly increase their use when other possibilities, such as mixture models, may be more directly translatable into a statistical test of the question at hand. In this sense, mixture models can be an alternative to ANOVA when the number of groups and the groups to which individuals belong are uncertain. Other clustering techniques may also be used, but may not be as appropriate or as useful (e.g. Fraley and Raftery, 1998; Steinly and Brusco, 2011). Simple mixture modelling also has the benefits of (1) yielding parameter estimates that can be used for inference (Fahey *et al.*, 2012), (2) being easily comparable using information theoretical methods, and (3) allowing for incorporation into more complex models.

This study is the first in a set of companion papers, in which we use mixture models of normal distributions to determine whether there is greater biological diversity than

currently recognized in populations of the salmonid fish, Arctic charr (*Salvelinus alpinus*), across lakes in Iceland. Arctic charr exhibit resource polymorphism, defined by the presence of discrete forms within a population that are associated with differences in resource use, yet are not geographically separated (Skúlason and Smith, 1995; Smith and Skúlason, 1996; Jonsson and Jonsson, 2001). Sympatric forms of Arctic charr may be differentiated in growth rates and maturity schedules (Griffiths, 1994; Klemetsen, 2010), morphology and diet (Malmquist *et al.*, 1992; Jónsson and Skúlason, 2000; Adams *et al.*, 2007), or reproductive and early life-history characteristics (Skúlason *et al.*, 1989, 1996; Adams *et al.*, 2006).

The database of the Ecological Survey of Icelandic Lakes (ESIL) project (Malmquist *et al.*, 2000; Kristjánsson *et al.*, 2011; Woods *et al.*, in press) presents a rare opportunity where information on intraspecific diversity of Arctic charr, within and among lakes, can be used to address important questions, such as how variation in environmental conditions might shape phenotypic distributions within and among lakes. In this study, we utilize the ESIL database to exemplify the process of detecting potential resource polymorphism using mixture models. To do this, we (1) collected available morphological and length-at-age data from 50 populations sampled in ESIL; (2) applied mixture models to these data to identify the most likely number of groups within lakes and assign individuals to the most likely groups; (3) discounted these groupings when sexual dimorphism was more likely to have caused them; (4) assessed whether phenotypic differences among putative groups were also related to maturity; and (5) analysed dietary patterns to confirm the hypothesis that phenotypic differences among groups constituted resource polymorphism. Finally, after classifying lakes as polymorphic, we analysed whether mixture models were more sensitive to detecting resource polymorphism at low levels of differentiation than general observation by experts (i.e. from anecdotal knowledge or documented studies).

MATERIALS AND METHODS

Arctic charr phenotypes

Two phenotypic attributes of Arctic charr were analysed for this study: length-at-age and body morphology. Fish were sampled once per lake during August to September in 1992–2004 as part of the ESIL project. In each of the 50 lakes (Table 1), 11 (22 for lakes > 10 km^2) single-mesh, single-strand nylon gill nets (Lundgren series) were set in the littoral zone from ~2 m depth outwards perpendicular to the shore and left overnight for 12 h. The gill nets were 25×1.5 m with mesh sizes 10, 12.5, 15.5, 19.5, 21.5, 24.0, 29.0, 35.0, 43.0, 55.0, and 60.0 mm, knot to knot. Photographs were taken and fork length was measured to the nearest 0.5 cm from all Arctic charr caught, and otoliths were removed from a subsample of up to 100 individuals for age determination. Otoliths were cleaned with a solution of methyl salicylate and annual growth rings were counted under a dissecting microscope. Individuals with no sign of gonadal development [stage 1 (Dahl, 1917)] were removed from the data set to reduce ontogenetic morphological trends within the data.

Morphological variables were derived from scanned images using a geometric morphometric approach in tps software [tpsDig v.2.1, tpsUtil v.1.40, tpsRel v.2.4 (Rohlf, 2004, life.bio.sunysb.edumorph)]. Eighteen landmarks and six sliding landmarks were placed at homologous locations on images of each fish (Fig. 1). Landmark configurations were unbent, aligned, rotated, and scaled to form a generalized orthogonal least-squares Procrustes average configuration. Partial warp scores for each individual were formed by

Table 1. Lakes from which Arctic charr were sampled for analyses are shown with their geographic coordinates, year sampled, ESIL database number, and the number of expected morphs according to expert knowledge (Exp. K)

Lake	Year	Lat. (N)	Long. (W)	Lake #	Exp. K	VB K	VB N1	VB N2	VB N3	VB~ Sex	Mat~ VB	D	M K	M N1	M N2	η^2	M~ Sex
Apavatn	1993	64°10′	20°38′	1	1	2	21	39	0	m, s	0.64	0.52	1	31	0	—	—
Elliðavatn	1993	64°05′	21°48′	2	1	1	51	0	0	—	—	—	1	35	0	—	—
Eyrarvatn	1992	64°25′	21°36′	3	1	1	59	0	0	—	0.54	—	1	35	0	—	—
Galtaból	1992	65°15′	19°43′	4	2	2	24	16	0	m	−0.14	0.882	1	29	0	—	—
Glammastaðavatn	1992	64°26′	21°35′	6	1	2	36	21	0	m	—	0.676	2	7	27	—	*s, m*
Hraunhafnarvatn	1993	66°31′	16°02′	7	1	1	39	0	0	—	0.96	—	1	37	0	—	—
Hvítárvatn	1994	64°36′	19°52′	8	2	1	57	0	0	—	—	—	1	31	0	—	—
Kötluvatn	1993	66°30′	16°30′	9	1	2	51	9	0	m	—	0.643	1	38	0	—	—
Langavatn	1993	64°07′	18°49′	10	1	2	31	20	0	m	—	0.681	1	27	0	—	—
Mjóavatn	1992	65°15′	19°48′	11	1	2	25	35	0	*s*	—	0.592	1	17	0	—	—
Selvatn	1992	65°57′	20°03′	13	1	1	39	0	0	—	—	—	1	37	0	—	—
Sigurðarstaðavatn	1993	66°29′	16°18′	14	1	1	46	0	0	—	—	—	1	45	0	—	—
Stóra-Viðarvatn	1993	66°14′	15°50′	17	3	3	18	24	13	m	−0.27	—	1	36	0	—	—
Svínavatn	1993	65°32′	20°06′	19	2	3	20	24	21	m, s	−0.23	—	1	60	0	—	—
Úlfljótsvatn	1993	64°06′	21°02′	20	2	2	63	16	0	m	−0.15	0.510	1	51	0	—	—
Vatnshlíðarvatn	1992	65°31′	19°38′	21	2	1	28	0	0	—	—	—	1	27	0	—	—
V-Friðmundarvatn	1992	65°18′	14°41′	22	1	2	63	12	0	m	—	0.394	1	18	0	—	—
Y-Deildarvatn	1993	66°24′	15°58′	23	1	2	19	17	0	m	−0.46	0.470	1	25	0	—	—
Ölvesvatn	1992	65°58′	20°05′	24	1	1	46	0	0	—	—	—	1	32	0	—	—
Haukadalsvatn	1994	65°35′	21°37′	28	2	2	45	14	0	m	—	0.862	2	18	28	—	*s, m*
Hítarvatn	1994	64°53′	21°55′	29	1	2	12	37	0	*n*	−0.36	0.546	1	43	0	—	—
Oddastaðavatn	1994	64°54	22°13′	30	1	1	39	0	0	—	−0.64	—	1	38	0	—	—
Vatnsholtsvatn	1994	64°49′	23°16′	31	1	1	44	0	0	—	—	—	1	34	0	—	—
Ánavatn	1994	65°13′	15°31′	32	1	2	19	38	0	m, s	—	0.731	1	34	0	—	—
Sænautavatn	1994	65°16′	15°31′	33	1	2	40	20	0	m	—	0.375	1	36	0	—	—
Eiðavatn	1994	65°24′	14°21′	34	1	1	25	0	0	—	—	—	1	24	0	—	—
Urriðavatn	1994	65°17′	19°51′	35	1	2	43	6	0	*n*	—	0.098	1	39	0	—	—

Thiðriksvallavatn	1995	65°41′	21°46′	36	1	—	—	—	—	—	—	—	1	40	0	—	—
Högnavatn	1995	65°48′	22°10′	37	2	3	28	10	41	m	−1.12	—	1	75	0	—	—
Ásbjarnarvatn-S	1996	65°03′	18°48′	39	1	2	38	47	0	m	—	0.382	2	13	37	—	*s, m*
Hópið	1996	65°31	20°30	41	1	2	22	36	0	m	—	0.366	—	—	—	—	—
Vesturhópsvatn	1996	65°28′	20°39′	42	1	1	59	0	0	—	—	—	1	37	0	—	—
Langavatn	1996	65°49′	17°17′	43	2	2	48	12	0	m, s	—	0.565	1	40	0	—	—
Reyðarvatn	1996	65°06′	18°32′	44	2	1	42	0	0	—	—	—	1	21	0	—	—
Fljótsbotn	1997	63°52′	18°54′	47	2	2	71	9	0	m	—	0.394	1	65	0	—	—
Frostastaðavatn	1997	64°01′	19°03′	48	1	3	18	17	24	*n*	—	—	1	34	0	—	—
E. Gíslholtsvatn	1997	63°57′	20°29′	49	1	1	59	0	0	—	—	—	1	58	0	—	—
Hestvatn	1997	64°01′	20°42′	50	2	2	33	37	0	m	—	0.639	1	64	0	—	—
Hlíðarvatn	1997	63°52′	21°43′	51	1	1	53	0	0	—	−0.33	—	1	51	0	—	—
Arnarvatn Stóra	1997	64°57′	20°19′	53	1	2	61	16	0	m	—	0.172	1	18	0	—	—
Úlfsvatn	1997	64°53′	20°35′	54	1	3	25	19	23	*s*	—	—	1	25	0	—	—
Langisjór	1998	64°10′	18°17′	57	1	1	20	0	0	—	2.07	—	1	17	0	—	—
Skorradalsvatn	1998	64°27′	21°09′	58	2	2	61	37	0	m, i	—	0.626	1	71	0	—	—
Lagarfljót	1998	65°10′	14°38′	59	2	2	51	67	0	m, s	—	0.457	1	29	0	—	—
Thuríðarvatn	1998	65°36′	15°10′	60	1	2	7	60	0	m	—	0.347	1	37	0	—	—
Heiðarvatn	1998	65°14′	14°10′	61	1	2	40	20	0	m	—	0.705	1	40	0	—	—
Skriðuvatn	1998	64°57′	14°38′	62	1	2	69	10	0	m, s	—	0.315	1	61	0	—	—
Sandvatn	1998	65°18′	14°41′	64	2	2	15	32	0	m, s	—	0.458	1	40	0	—	—
Thríhyrningsvatn	1998	65°10′	15°46′	65	2	1	62	0	0	—	−0.51	—	2	31	19	0.660	m, s
Hafravatn	1998	64°07′	21°44′	67	2	1	12	0	0	—	—	—	—	11	0	—	—
Total lakes $K > 1$	—	—	—	—	17	31	—	—	—	—	—	—	4	—	—	—	—

Note: The number of groups (1–3) detected in the best-fit model according to AICc is shown for each population tested with Von Bertalanffy growth curve models (VB K) and bivariate morphology models (M K). The numbers of individuals placed in each group are indicated by the corresponding N1, N2, and N3 columns, which are labelled according to the corresponding coefficient estimates in Tables S1 and S2 (evolutionary-ecology.com/data/2751Appendix.pdf). Differentiation (D) and Effect Sizes (η^2) were calculated for lakes that yielded $K = 2$ in growth curve (VB K) and morphology (M K) tests, respectively. Results from logistic regressions to predict maturity based on residuals of single-curve VB models are summarized as coefficients from significant models (Mat~VB). Results from ANOVAs to predict (1) residuals of single-curve VB models (VB~Sex*Morph) and (2) morphology (M~Sex*Morph) based on sex and morph assignment are summarized by listing significant effects in order of their effect sizes (m = morph assignment, s = sex, i = interaction, n = none). Italicized effects indicate that polymorphic status was removed from these lakes due to non-significance of morph assignment or a greater effect size of sex.

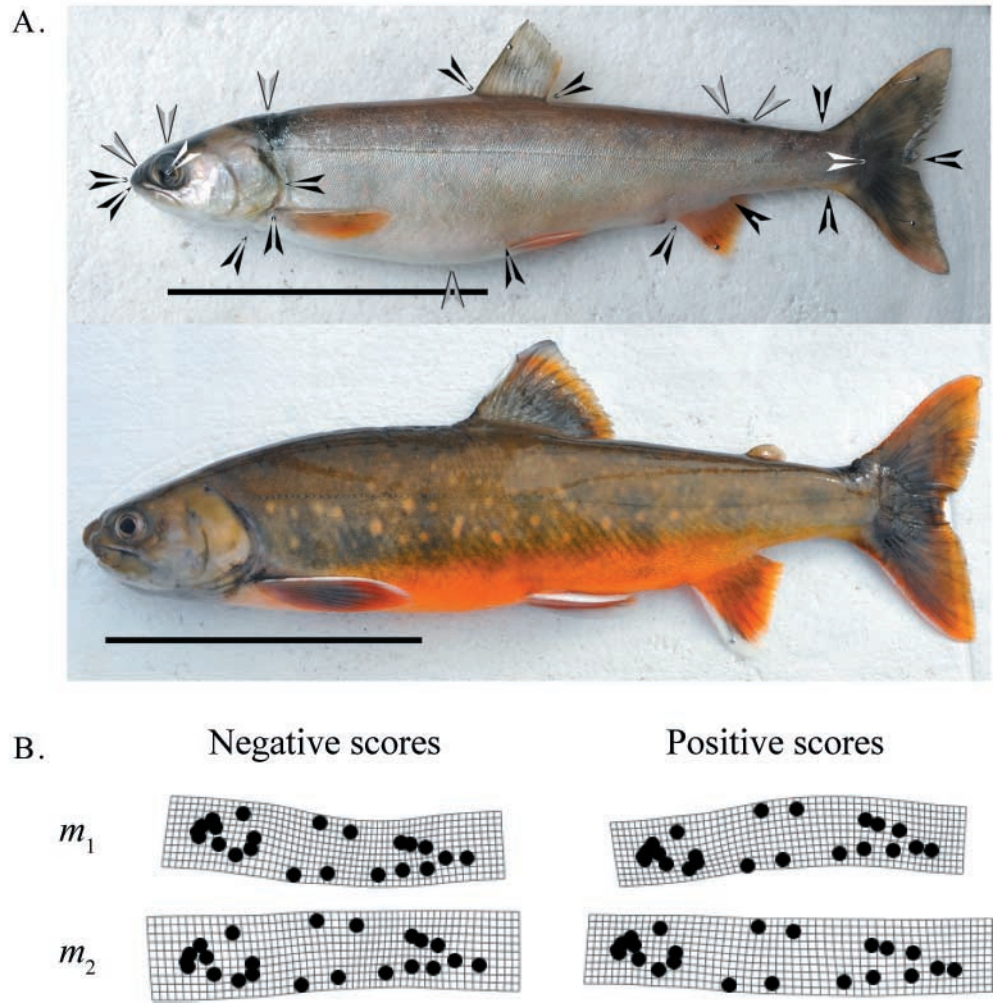

Fig. 1. (A) Depicted Arctic charr (*Salvelinus alpinus*) individuals are planktivorous (limnetic form, top panel) and benthivorous (large benthic form, bottom panel) respectively. They are from Thingvallavatn, which also contains a piscivorous and a small benthic morph (Snorrason and Skúlason, 2004). The 12 black and two white arrows in the upper panel represent fixed landmarks; the six grey arrows indicate sliding landmarks. Black bar = 10 cm. (B) Points correspond with landmark positions in (A) and are deformation grid visualizations of extreme scores within the range of data. Low scores on the first axis reflected ventral bending in the abdomen, deeper heads, shorter caudal peduncles, and a more upturned mouth (m_1, top left), whereas high m_1 scores yielded the opposite (m_1, top right). The second axis yielded short bodies and caudal peduncles with large heads (m_2, bottom left) versus the opposite (m_2, bottom right).

comparing the relative departure in configuration of individuals from the average configuration, and analysed with a relative warp analysis. A relative warp analysis is analogous to a principal component analysis and results in scores on orthogonal relative warp axes for each individual that can be reflected in deformation grids (Bookstein, 1991; Rohlf, 2004). The first two

relative warps were used as morphological variables (m_1, m_2). Relative warps were then tested for effects of allometry by regressing them over fork length.

Detecting polymorphism

In some cases polymorphism may be revealed by distinct growth curves or morphologies, but in others it may be difficult to distinguish between different phenotypes. In these difficult cases we sought objective criteria by which broad variability in biological attributes could be distinguished from the presence of overlapping groups. To do this, we first collected expert knowledge from published reports and ESIL survey data (Jónsdóttir *et al.*, 1998; Jónsson and Skúlason, 2000; Jónsson, 2002; Jónsson and Malmquist, 2002; Malmquist, 2004; Wilson *et al.*, 2004) that indicated the presence of forms that appear morphologically different or grow to and mature at small sizes (~15 cm) in the presence of larger Arctic charr (Table 1). This knowledge was used as a baseline with which to compare results gained from the fitting of mixture models to Arctic charr biological attributes. Statistical evidence for the presence of polymorphism was then evaluated by choosing the best model with the lowest Akaike's Information Criterion corrected for small sample sizes (AICc) among models that included K components. These evaluations included a comparison among (1) a growth curve model containing either 1, 2 or 3 Von Bertalanffy growth functions, and (2) unimodal, bimodal, and trimodal multivariate normal distributions fit to bivariate morphological data. Although it remains controversial which criteria are the best for recovering the actual number of groups present in mixture distributions, AIC shows promise (Cubaynes *et al.*, 2012).

To perform the first evaluation, i.e. among growth curve models, a model with only a single Von Bertalanffy (VB) growth function (equation 1) was fit as:

$$\mu = L_{\infty}\left(1 - e^{(-\kappa a)}\right) + \varepsilon \tag{1}$$

where length (μ) was predicted using a = age data, L_{∞} = asymptotic length parameter, and κ = curvature parameter. Normal errors were assumed (i.e. $\varepsilon \sim N(0, \sigma)$), so the sum of the negative log likelihoods from a normal probability density function was minimized to estimate the three parameters (L_{∞}, κ, σ). Two separate VB functions were then fit to the length data (x) by minimizing the negative log of the likelihood function (L) based on a mixture of two normal density functions:

$$L = \Pi\left(p \frac{1}{\sqrt{2\pi\sigma_1^2}} e^{-\frac{x-\mu_1}{2\sigma_1^2}} + (1-p) \frac{1}{\sqrt{2\pi\sigma_2^2}} e^{-\frac{x-\mu_2}{2\sigma_2^2}}\right) \tag{2}$$

where μ_1 and σ_1 are parameters defining the first normal distribution in the mixture, μ_2 and σ_2 are parameters defining the second normal distribution, and p is the proportion that the first distribution contributes to the mixture. In this case, μ_1 and μ_2 in equation (2) are defined by two separate VB functions, each with a set of parameters (L_{∞}, κ). The likelihood of a single data point is therefore a sum of the likelihoods that it came from each distribution, weighted by the proportional contribution of that distribution. This model represents the situation in which multiple morphs co-exist within a population. Assignment of individual data points to one of the growth curves can be made by calculating what proportion of that individual's total likelihood was attributable to each distribution (and corresponding growth curve). These are the posterior probabilities, and individuals can then be assigned to

the growth curve with the maximal posterior probability (McLachlan and Peel, 2000). This model was then extended to fit three growth curves:

$$L = \Pi\left(p_1 \frac{1}{\sqrt{2\pi\sigma_1^2}} e^{-\frac{x-\mu_1}{2\sigma_1^2}} + p_2 \frac{1}{\sqrt{2\pi\sigma_2^2}} e^{-\frac{x-\mu_2}{2\sigma_2^2}} + (1 - p_2 - p_3) \frac{1}{\sqrt{2\pi\sigma_3^2}} e^{-\frac{x-\mu_3}{2\sigma_3^2}}\right) \quad (3)$$

where p_2 is the proportion that the second distribution contributed to the mixture, and μ_3 and σ_3 are parameters for the third normal distribution as defined by the fit of a third VB function. Example code used in the R statistical software v. 2.13.0 (R Development Core Team, 2011) is provided in the Appendix (evolutionary-ecology.com/data/2751Appendix.pdf).

Polymorphism in morphology was detected within each lake by assuming multivariate normal error for morphology (i.e. $\varepsilon \sim \text{MVN}(\boldsymbol{\mu}, \mathbf{E})$), where $\boldsymbol{\mu}$ is a vector of morphological variable means (e.g. $\boldsymbol{\mu} = (\bar{\mu}_1, \bar{\mu}_2)$) and $\mathbf{E}$ is the covariance matrix, and their dimensions depend on the number of morphological variables included. Multivariate normal distributions were chosen because preliminary analyses indicated that univariate distributions of morphological variables were close to normal, with informative deviations in some lakes. A unimodal model of morphology ($K = 1$) was fit simply by estimating these parameters within $\text{MVN}(\boldsymbol{\mu}, \mathbf{E})$, whereas a bimodal model was fit by estimating a mixture model of two multivariate normal distributions. For a bimodal mixture model ($K = 2$), the parameters included were two separate vectors of means ($\boldsymbol{\mu}$), two covariance matrices ($\mathbf{E}$), and a proportional contribution p of the first multivariate distribution [i.e. the same as equation (2) except univariate density functions were extended to be multivariate density functions]. Trimodality ($K = 3$) in morphology was fit using a mixture model of three multivariate normal distributions [i.e. three separate vectors of means ($\boldsymbol{\mu}$), three covariance matrices ($\mathbf{E}$), and two proportional contributions p_1 and p_2 of the first and second multivariate distributions respectively, similar to equation (3)]. The package mixtools v.0.4.4 in the R statistical software v.2.13.0 was used to fit unimodal multivariate normal distributions and finite mixtures of two or three multivariate normal distributions with an EM algorithm (Benaglia *et al.*, 2009). All other analyses were performed using base packages (R Development Core Team, 2011).

Fitted models with highly uneven numbers of members between groups tended to yield higher likelihoods than models with more even groups. Because we found this result difficult to interpret biologically, we restricted our evaluations of polymorphism to those models that yielded groups having more than five members. Starting values of morphological models were randomly chosen 30 times and models that minimized the corrected Aikaike's Information Criterion (AICc) were retained, with the constraint that each resulting group contained more than five members. To assess the relative contributions of m_1 and m_2 to the formation of bivariate groupings, we then analysed each separately as a single variable to determine whether bimodal univariate distributions fit better than unimodal distributions.

Analysing effects of maturity and sexual dimorphism

Maturity is often used to distinguish between morphs in polymorphic systems; however, it is closely linked physiologically with body growth. Therefore, logistic regressions were used to determine whether the relationship between growth and maturity was similar between polymorphic and monomorphic populations. Individuals exhibiting no gonadal development were excluded from the analyses, so that any difference in timing of sexual maturity

could not be interpreted solely as a difference in frequency of adults versus juveniles. Early stages of maturation were counted as 2–3 on the scale of Dahl (1917) to reflect slight development, whereas late stages were counted as 4–6 (ripening to spawning) to reflect imminent spawning. Residuals taken from the single-curve VB models fit in the previous analysis were used to indicate individual variation in growth and to predict maturity using a binomial error distribution and logit link function. Where these models were significant, coefficients were reported: negative coefficients indicated a greater likelihood of maturity at small sizes. Coefficients were then subjected to a Student's *t*-test to determine whether they differed between monomorphic and polymorphic populations.

The effect of sex was explored using ANOVAs to predict (1) residuals from the single VB-curve model (i.e. individual growth) and (2) morphology variables using sex, morph assignment, and their interaction as predictors. When morph assignment had stronger effects than sex, we concluded that the current groupings were due to polymorphism rather than sexual dimorphism. Interactions were removed when non-significant.

Confirming an association of polymorphism with resource use

To confirm that the best-fit models indicating polymorphism were related to resource use, we first reduced the dimensionality of the dietary count data by performing a detrended correspondence analysis (DCA) using the package vegan v.2.0-3 (Oksanen *et al.*, 2012) in the R statistical software v.2.13.0. Only certain categories were included that reflected major components of Arctic charr diets and habitat-associated feeding behaviours, including: snails (*Radix peregra*); chironomid larvae; chironomid pupae; tadpole shrimp (*Lepidurus arcticus*); pea clams (*Pisidium* spp.); the cladocerans *Bosmina* spp., *Alona* spp., *Daphnia* spp., and *Eurycercus* spp.; and the threespine stickleback *Gasterosteus aculeatus*. Woods *et al.* (in press) provide details on gut content analyses completed as part of the ESIL project. Scores on four resulting axes from this analysis were then predicted using categorical morph assignments in linear models to test for differences in diet among morphs. *P*-values were adjusted within lakes using a sequential Bonferroni correction.

Calculating differentiation

Only the presence of polymorphism can be detected in the above analysis, whereas strength of polymorphism could be reflected by the degree of overlap between the two forms. For morphology, the measure Effect Size (η^2) can be used to reflect relative overlap. The variable with the greater reduction in AICc in univariate analyses of morphology was used to calculate η^2 as the sum of squared errors due to treatments or groups (between-treatment error) divided by the total sum of squares. However, because our growth curve analysis was based on a non-linear model, this ANOVA-related definition is inappropriate. We therefore describe a metric of Differentiation (D) below that we defined to be similar to Effect Size.

If the two-curve fit is taken to be the presence of a treatment ($K = 2$), and a one-curve fit is taken to be the absence of a treatment ($K = 1$), then a measure similar to the ANOVA-based 'between-treatment error' can be calculated as the sum of the squared differences between individual predictions from the two-curve model and from the one-curve model. If the total sum of squares term used in ANOVA is then taken as the sum of the squared residuals from the single-curve model ('total error'), dividing the sum of the squared between-curve differences (i.e. 'between-treatment error' term) can be divided by this 'total error' to yield

a measure similar to Effect Size (referred to as 'Differentiation'). Because predictions are necessary for calculating Effect Size and Differentiation (D), individuals were first assigned to the most likely growth curve using the posterior probabilities described in the previous section. This measure was only calculated for lakes with $K = 2$ due to the difficulty in interpreting the relative values of these measures between lakes with $K = 2$ versus $K = 3$.

Sensitivity of mixture models versus expert knowledge to differentiation

To determine whether mixture models detected more polymorphic systems with lower differentiation values than expected according to expert knowledge, three analyses were conducted. First, the presence of polymorphism across all 50 lakes was predicted using D in a logistic regression with Bernoulli errors and a logit link function. In this case, the predicted variable, presence of polymorphism, was indicated by significance in the ANOVAs used to confirm the relationship between polymorphism and resource use. Second, the same model was used, but the presence of polymorphism (predicted variable) was based on expert knowledge, and results were compared with the first model. Finally, values for D were compared using a Student's t-test between lakes categorized as polymorphic by (1) expert knowledge and (2) mixture models. This last analysis excludes all lakes that did not show a bimodal distribution (i.e. monomorphic populations and polymorphic populations with $K = 3$), and may have the same D values in both categories when a lake was categorized as polymorphic by both criteria (i.e. expert knowledge and mixture model results).

RESULTS

Arctic charr phenotypes

In total, 2796 fish from 50 populations with length-at-age data, 12–118 per population, were included in growth curve analyses. For morphological analyses, 1873 fish were included from 50 populations (49 the same lakes as in the growth curves analyses), 11–71 per population. The first relative warp axis (m_1) accounted for 24.2% of the total variation and reflected bending around the abdominal section (likely resulting from variation in the placement of fish), as well as variation in head depth and caudal peduncle length. Although the bending has little biological meaning, the associated variation in head and caudal peduncle shape may be interpreted biologically. Negative m_1 scores also indicated expansion of the lower head and a more upturned mouth, whereas positive m_1 scores yielded an expansion of the upper head and a more downturned mouth. Therefore, we cautiously use this relative warp as a morphological variable in further analysis, although the alternative explanation of being attributable to bending is considered in our interpretation of the results. The second relative warp axis (m_2) accounted for 15.6% of the total variation; individuals with positive scores had larger heads and shorter bodies, especially in the caudal peduncle region (Fig. 1).

In regressions of relative warps over fork length, neither m_1 nor m_2 showed a significant effect of allometry (m_1: $F_{1,1986} = 0.040$, $P = 0.842$; m_2: $F_{1,1986} = 1.756$, $P = 0.185$). Therefore, analyses proceeded with original relative warp score values.

Detecting polymorphism

According to expert knowledge, only 17 populations were thought to be polymorphic, whereas the mixture model comparisons detected $K > 1$ in 32 of 50 populations (48 tested with both growth and morphology data; one tested with only growth and one tested with only morphology data; Table 1). Of the 17 populations thought to be polymorphic by expert knowledge, 13 were detected as having $K > 1$. Of the 32 populations with evidence of polymorphism ($K > 1$), 28 were supported with only growth models (Fig. 2); in one population, polymorphism was supported with only the morphological model (Fig. 3); and in three populations, polymorphism was supported with both tests. Of the 31 populations

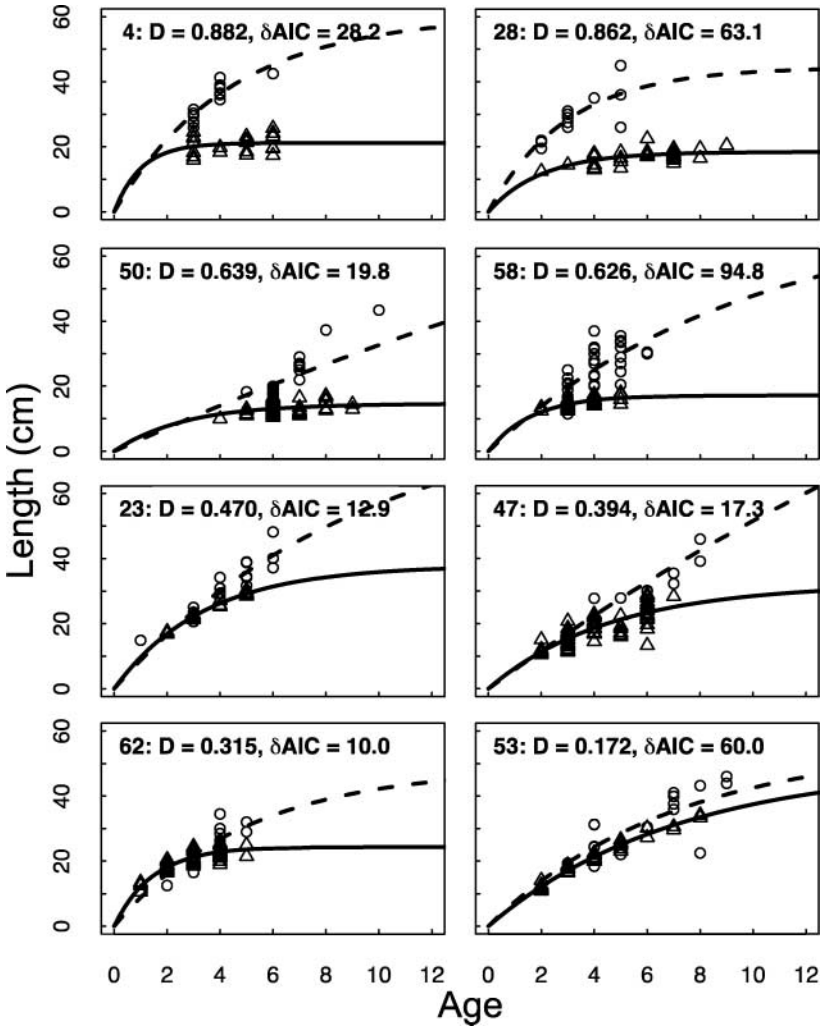

Fig. 2. Examples showing two-growth curve Von Bertalanffy model fits ($K = 2$). Differentiation (D) and change in AICc (δAIC) are shown with lake database numbers (Table 1). Circles are individuals assigned to the dashed growth curve; triangles are individuals assigned to the solid growth curve. Panels are ordered as examples of low to high D moving upward.

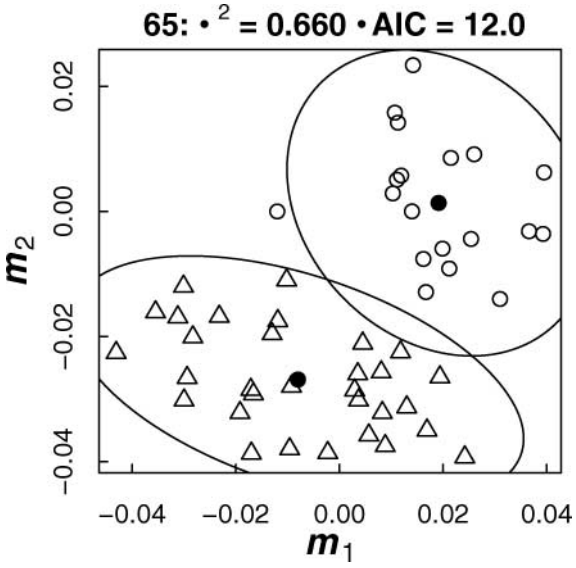

Fig. 3. Bivariate morphology bimodal model fit ($K = 2$) for Thríhyrningsvatn (Lake 65). Effect Size (η^2) and change in AICc (δAIC) are indicated (Table 1). Open circles and triangles distinguish the morph assignments, solid circles indicate mean values for each morph, and ellipses delineate the 95% confidence intervals for each bivariate normal density function. Axis labels (m_1, m_2) refer to the first and second relative warp, respectively.

for which polymorphism was supported with growth models, a model with three growth curves had the best fit in five populations, whereas two growth curves were best for the other 26 populations. All four morphological evaluations yielding support for polymorphism indicated that a bimodal model fit best. Mixture model parameter estimates and standard errors for individual populations can be found at evolutionary-ecology.com/data/2751Appendix.pdf (Tables S1 and S2).

Analysing the effects of maturity and sexual dimorphism

In the logistic regressions predicting maturity status based on deviation from a single curve model, 14 lakes showed significant results. Coefficients indicated the strength and direction of the link between growth and maturation for each lake (Table 1, column Mat~VB), and full model results can be seen at evolutionary-ecology.com/data/2751Appendix.pdf (Table S3). For both monomorphic and polymorphic populations, most coefficients were negative, indicating that slower growing individuals matured earlier in most cases. The *t*-tests of coefficient values between monomorphic and polymorphic populations were not significant, although polymorphic populations appeared to have a slightly greater probability of maturing at small sizes (coeff. = −0.809, S.E. = 0.406, $t = -1.992$, $P = 0.070$).

In the ANOVAs using sex, morph assignment, and their interaction to predict residuals from a single-curve VB model, two lakes in the former analysis showed a stronger effect of sex than morph assignment and three lakes showed a lack of significance of these models. Therefore, the polymorphic designation of these five lakes was removed and mixture model results were either attributed to sexual dimorphism or considered too weak to analyse

further. In the ANOVAs using sex, morph assignment, and their interaction to predict morphology, three of the four lakes had stronger effects of sex than morph assignment (Table 1). However, these did not lose their polymorphism designation because the VB results still indicated a strong morph effect, so further analyses relied only on the VB results. The fourth lake showed a stronger effect of morph assignment than sex. The relative strengths of effects are indicated in Table 1 and full ANOVA tables can be found at evolutionary-ecology.com/data/2751Appendix.pdf (Tables S4 and S5). Reductions from these analyses left 27 lakes to be confirmed as exhibiting resource polymorphism using diet data.

Confirming an association of polymorphism with resource use

The detrended correspondence analysis yielded four axes, the first of which reflected variation between consumption of fish and pelagic cladocerans (positive scores) versus snails (negative scores) (Table 2). The second axis reflected variation in consumption of tadpole shrimp (positive) versus pea clams (negative), the third axis reflected variation in consumption of fish (positive) versus benthic cladocerans (negative), and the fourth reflected variation in consumption of the benthic predatory cladoceran *Eurycercus* spp. (positive) versus chironomid pupae (negative).

Fourteen of the 27 populations showed significant differences in diets between morph groups along at least one of the four DCA axes in linear models at $P < 0.5$ (after a sequential Bonferroni correction). Three additional lakes showed marginally significant differences at $P < 0.1$. Two additional lakes did not show significant differences in resource use in this study, but were considered confirmed by diet analyses presented in other studies (Jónsson, 2002; Jónsson and Malmquist, 2002), as sample sizes were small relative to these other studies. A summary of these linear model results can be seen in Table 3, but a table of full results is available at evolutionary-ecology.com/data/2751Appendix.pdf (Table S5).

Table 2. The detrended correspondence analysis (DCA) of gut content count data yielded four axes that were used in further analyses to determine whether putative morphs differed in resource use

	DC1	DC2	DC3	DC4
Eigenvalues	*0.8914*	*0.7618*	*0.8069*	*0.7285*
Radix peregra	**–1.543**	–0.160	–0.094	–0.224
Pisidium spp.	–0.898	**–1.622**	0.337	–0.510
Lepidurus arcticus	–0.849	**1.755**	0.150	–0.569
Eurycercus spp.	–0.498	0.441	**–1.246**	**1.334**
Alona spp.	**1.516**	–0.386	**–1.493**	0.267
Bosmina spp.	**1.788**	0.610	**1.105**	–0.800
Daphnia spp.	**2.287**	0.183	–0.311	0.297
Chironomid larvae	–0.054	–0.198	0.974	0.600
Chironomid pupae	0.313	0.141	–0.720	**–1.326**
Fish	**1.160**	0.232	**1.726**	0.719

Note: Loadings of dietary items are shown with values > 1 highlighted in bold and contributed substantially to the indicated axis.

Table 3. Results are shown for linear models to predict differences in diet based on detrended correspondence analysis factor scores (DC1–DC4)

			DC1				DC2				DC3				DC4				
L	Model	d.f.	Coeff.	S.E.	*t*	*P*	Coeff.	S.E.	*t*	*P*	Coeff.	S.E.	*t*	*P*	Coeff.	S.E.	*t*	*P*	Confirmed
1	VB	34	0.113	0.114	0.991	0.329	0.071	0.082	0.862	0.790	0.126	0.172	0.734	1.000	0.002	0.005	0.488	1.000	
4	VB	20	0.578	0.596	0.971	0.343	0.084	0.086	0.971	0.687	0.389	0.401	0.971	1.000	0.202	0.208	0.971	1.000	*
6	VB	35	0.254	0.173	1.471	0.300	0.239	0.132	1.808	0.079	−0.057	0.213	−0.270	1.000	−0.088	0.299	−0.294	1.000	?
9	VB	38	0.269	0.287	0.936	1.000	−0.066	0.055	−1.199	0.713	−1.115	0.356	−3.136	*0.003*	−0.388	0.292	−1.328	0.384	✓
10	VB	31	−0.434	0.201	−2.161	0.116	0.011	0.039	0.287	1.000	−0.310	0.141	−2.190	0.072	−0.234	0.100	−2.342	*0.026*	✓
17	VB	43	1.097	0.439	2.499	*0.049*	0.839	0.163	5.147	<0.001	−0.190	0.187	−1.014	0.100	0.422	0.159	2.644	*0.023*	✓
19	VB	57	2.544	0.269	9.461	*0.000*	0.158	0.050	3.184	*0.007*	−0.254	0.126	−2.026	0.190	0.397	0.076	5.238	*<0.001*	✓
20	VB	63	1.076	0.324	3.319	*0.002*	0.057	0.086	0.660	1.000	−0.345	0.200	−1.730	0.177	0.120	0.158	0.758	1.000	✓
22	VB	25	−0.684	0.223	−3.068	*0.005*	−0.235	0.161	−1.456	0.631	−0.614	0.394	−1.559	0.395	−0.650	0.284	−2.290	0.061	✓
23	VB	32	−0.317	0.207	−1.535	0.404	−0.022	0.063	−0.357	1.000	0.197	0.115	1.715	0.192	−0.097	0.046	−2.104	*0.043*	✓
28	VB	39	−0.839	0.260	−3.230	*0.003*	−0.136	0.048	−2.871	*0.013*	0.140	0.220	0.638	1.000	0.384	0.224	1.716	0.283	✓
32	VB	27	0.208	0.297	0.701	1.000	−0.159	0.093	−1.704	0.200	−0.141	0.184	−0.768	1.000	−0.261	0.125	−2.083	*0.047*	✓
33	VB	31	−0.413	0.290	−1.425	0.493	−0.051	0.045	−1.150	1.000	−0.316	0.198	−1.596	0.121	−0.176	0.117	−1.496	0.289	
37	VB	75	0.214	0.081	2.639	*0.010*	0.125	0.172	0.725	1.000	−0.133	0.164	−0.813	1.000	0.182	0.189	0.964	0.676	✓
39	VB	64	0.126	0.146	0.868	0.777	0.155	0.089	1.736	0.087	−0.055	0.077	−0.717	1.000	−0.084	0.131	−0.640	1.000	?
41	VB	30	−0.536	0.493	−1.087	0.572	−0.040	0.062	−0.650	1.000	0.390	0.274	1.426	0.164	0.194	0.211	0.918	1.000	
43	VB	29	−0.014	0.491	−0.029	1.000	0.086	0.134	0.642	1.000	−0.378	0.622	−0.608	1.000	−0.238	0.324	−0.735	0.468	
47	VB	65	−0.054	0.169	−0.318	0.565	−0.151	0.084	−1.807	0.151	0.404	0.316	1.278	0.617	0.699	0.249	2.805	*0.007*	✓
50	VB	50	0.167	0.246	0.680	1.000	0.066	0.054	1.233	*0.050*	−0.273	0.144	−1.903	*0.004*	−0.279	0.154	−1.817	*0.006*	✓
53	VB	18	−0.604	0.473	−1.277	0.435	−0.124	0.248	−0.499	1.000	−1.023	0.376	−2.719	*0.014*	−0.002	0.275	−0.009	1.000	✓
58	VB	85	1.488	0.217	6.869	*<0.001*	0.454	0.042	10.805	*<0.001*	0.281	0.143	1.962	0.212	−0.793	0.114	−6.941	*<0.001*	✓
59	VB	69	0.112	0.164	0.681	1.000	−0.037	0.026	−1.425	0.159	0.124	0.123	1.008	0.951	0.128	0.112	1.149	0.509	
60	VB	34	0.069	0.163	0.422	1.000	−0.148	0.171	−0.864	0.394	−0.075	0.213	−0.353	1.000	−0.034	0.204	−0.166	1.000	
61	VB	25	0.076	0.065	1.183	0.248	−0.029	0.051	−0.574	1.000	−0.156	0.228	−0.682	1.000	−0.039	0.241	−0.160	1.000	
62	VB	51	−0.082	0.182	−0.452	1.000	0.251	0.215	1.167	0.249	0.044	0.282	0.157	1.000	−0.013	0.294	−0.045	1.000	
64	VB	31	0.148	0.348	0.427	1.000	0.210	0.352	0.596	1.000	−0.088	0.255	−0.345	1.000	0.118	0.160	0.739	0.465	
65	M	70	0.186	0.322	0.578	1.000	0.157	0.168	0.933	0.354	0.100	0.140	0.712	0.958	−0.045	0.091	−0.490	1.000	*

Note: Only populations with best-fit models that supported the presence of polymorphism (K > 1) were subjected to tests. Lake ESIL number (L) is indicated, together with which model supported the presence of polymorphism: mixture models of multiple growth curves (VB) or mixture models of bivariate morphological distributions (M). Italicized lake numbers were expected to be polymorphic based on expert knowledge. Estimates for the coefficient for morph assignment, standard errors (S.E.), *t*-values, and *P*-values are shown. All *P*-values have been corrected within lakes using sequential Bonferroni adjustment. A check (✓) in the Confirmed column indicates that support was found for resource polymorphism at $P < 0.5$, whereas a question mark (?) indicates marginal support at $P < 0.1$. *P*-values in italics indicate $P < 0.05$. The asterisk (*) for Lakes 4 and 65 indicates that this assessment is not supported by our analysis but known dietary differences have been documented previously (Jónsson, 2002; Jónsson and Malmquist, 2002).

Sensitivity of mixture models versus expert knowledge to differentiation

Of 50 lakes tested, polymorphism was present in 17 lakes according to expert knowledge, whereas it was present in 16 lakes according to mixture model methods. Logistic regressions indicated that the probability of classifying a lake as exhibiting resource polymorphism marginally increased with its D value for both mixture model methods (coeff. = 4.424, S.E. = 2.533, $Z = 1.747$, $P = 0.0807$) and expert knowledge (coeff. = 4.525, S.E. = 2.639, $Z = 1.715$, $P = 0.0864$). Coefficients of these two models were similar. The value for D also did not differ between lakes confirmed as exhibiting resource polymorphism and those classified by anecdotal or published knowledge by experts (coeff. = −0.016, S.E. = 0.078, $t_{21} = -0.203$, $P = 0.841$). Therefore, there appears to be little difference in sensitivity of mixture models versus expert anecdotal knowledge to level of differentiation for the detection of resource polymorphism.

DISCUSSION

This study has shown that mixture models are useful for evolutionary and ecological studies to detect intraspecific multimodal diversity. These models detected resource polymorphism in Arctic charr populations spanning a wide geographical range throughout Iceland, even when differentiation between groups appeared low. Gaining an understanding of intraspecific diversity across systems can greatly improve comparative ecological studies, as the functional role of a polymorphic species may change drastically across locations, even if its species designation does not. Few comparative studies on resource polymorphism have been completed (but see Griffiths, 1994; Landry *et al.*, 2007; Siwertsson *et al.*, 2010), despite their importance for gaining a broader understanding of processes contributing to the evolution and ecology of species.

Resource polymorphism in Arctic charr across Iceland

We found evidence for polymorphism in 13 of the 17 populations expected to be polymorphic based on expert knowledge, the exceptions being populations within Hvítárvatn, Vatnshlíðarvatn, Reyðarvatn, and Hafravatn (Table 1). Hafravatn likely had too small a sample ($N = 12$) and was excluded from morphological analyses for this reason. The others, especially Vatnshlíðarvatn and Reyðarvatn ($N = 28$ and 42, respectively), may have had inadequate or not fully representative samples, as most other polymorphic populations had $N > 40$. For example, a narrow range of observed ages can reduce the fit of multiple growth curves. Alternatively, for populations that have been shown in previous studies to be morphologically differentiated [e.g. Vatnshlíðarvatn (Jónsson and Skúlason, 2000)], the informative morphological traits may not have been captured by the morphological variables used in this study (e.g. colour). This may also explain why fewer populations of Arctic charr were found to diverge in morphology than growth; only one population had support for polymorphism based entirely on morphological analyses (Thríhyrningsvatn). Nineteen populations detected as polymorphic in growth were not believed to be polymorphic based on expert knowledge; however, only six of these gained support at $P < 0.05$ from diet patterns. Possibly the polymorphic populations that show no indication of differences in resource use contain other forms of individual heterogeneity, such as inter-cohort growth variation (Borchering *et al.*, 2010). However, although a Bonferroni correction was applied

to diet analyses within lakes, no correction was applied across lakes, so 1–2 of our lakes may have been misclassified.

The polymorphic status of five lakes (of the 19 assigned $K > 1$ based on VB models only) was removed due to a lack of significance in morph assignment or a strong effect of sex in ANOVAs. This approach allows our method to be at least as conservative as methods such as ANOVA, traditionally used to detect polymorphism. Although polymorphic populations appeared to have more individuals maturing early at smaller sizes, the lack of significance indicates that this is not uncommon in monomorphic lakes also. Therefore, although differences in timing of maturity are frequently used as evidence of morph differentiation in polymorphic systems, it is difficult to distinguish this from monomorphic variability in individual growth rate. In some cases (e.g. in the case of large recruitment cohorts), juvenile growth rate and even age at maturity can change significantly (Snorrason *et al.*, 1994).

The sensitivity of our analyses to the relative differentiation between morphs was surprisingly similar to that of expert knowledge – both were generally insensitive to the level of differentiation. Despite this, those cases of polymorphism likely to be published may be biased towards more clear-cut cases. The presented methods, therefore, may be useful as a framework for comparing systems in a standardized manner. Other indices reflecting the degree of overlap among groups may also prove useful [e.g. overlapping area MI, sample means quotient, or overlapping area between two normal distributions, etc., *sensu* Ipiña and Durand (2010)].

Caution in the use of mixture models

The purpose of this study was to present a simple but detailed explanation of how mixture models may be useful in answering some of the most fundamental questions in ecology and evolutionary biology, not to present a comprehensive review of methods involving mixture models (see instead Fraley and Raftery, 1998; McLachlan and Peel, 2000; Royle, 2004; Benaglia *et al.*, 2009; Reinecke and Seddig, 2011; Steinly and Brusco, 2011). Mixture models are appropriate and useful tools for a wide variety of ecological and evolutionary studies but we emphasize a few important practical considerations when using such models. First, problems may arise in the fitting of mixture models due to the presence of many local maxima and the need for reasonable starting values; however, using the EM algorithm eases some of these problems (McLachlan and Peel, 2000). Spurious groupings are also possible, and the best approach for selecting models with the true number of components remains controversial (McLachlan and Peel, 2000).

Second, as with any statistical analysis, the detectability of the mixture model method is sensitive to sample sizes, and especially differences in sample size between groups. However, unlike standard ANOVA methods frequently used to distinguish between groups designated *a priori*, this method assigns individuals to groups after defining the best mixture model. Therefore, it can be sensitive to outliers for the same reason that outliers are designated as such: they do not fit the assumed normal distribution. When using an ANOVA, outliers are frequently removed, but in this analysis they may be designated as their own group. In particularly variable natural populations, oddities are perhaps more common than would be expected from a theoretical distribution, although this may not reflect the presence of an entire additional form, as expected from polymorphism. Unfortunately, it may be impossible to distinguish between the presence of oddities versus insufficient sampling of a second group. For example, uneven sampling may result when catch rates differ among morphs due to differences in habitat or body size (Finstad *et al.*, 2000). Considering the effort

needed to gain enough data to do broad-scale comparative analyses, uneven sampling is likely to occur, so over-classification as polymorphic may be a persistent problem when using these methods. We avoided this problem by using a minimum number of individuals assigned per component (> 5), but this may not be appropriate in other studies. Therefore, this problem must be dealt with on an individual basis and, as with the removal of outliers, is up to the discretion of the researcher.

Third, other problems are inherent during the interpretation of statistical results as biologically meaningful, although these would also likely apply to any statistical method. For example, the emphasis in our study was placed on detecting the presence of bimodal or trimodal distributions over a unimodal distribution with a large variance. However, both multimodal distributions and unimodal distributions with large variances may be biologically meaningful in understanding how populations transition from monomorphic to polymorphic states (Bolnick *et al.*, 2003). In addition, differentiation was used in this study only to characterize bimodal populations due to the problem of defining a measure that would be meaningful in both bimodal and trimodal populations. As the underlying biological mechanisms causing bimodal versus trimodal populations remain unclear, a measure reflecting the strength of this phenomenon would be difficult to define. This emphasis on discrete forms indicates that individuals may be assigned to individual forms, although in reality the strength of this polymorphism may vary on a continuum from vague ecological differences to strong phenotypic differences reinforced by reproductive isolation. Furthermore, although polymorphic populations are interpreted here as being temporally stable, some populations with weaker differentiation may actually reflect environmental variation or groups of cohorts with different growth rates or morphologies. Population dynamical mechanisms may cause bimodality to develop within a population due to a combination of seasonal effects of different-sized prey and size-specific foraging (Griffiths, 1994; Borchering *et al.*, 2010).

Finally, our choice in using mixtures of normal distributions reflects an assumption that multiple groups within our sample were normally distributed with separate parameters describing their distributions (e.g. means, and therefore by definition modes). Mixture models are generally so flexible that any non-normal distribution can be reasonably approximated by a finite mixture of normal distributions (McLachlan and Peel, 2000). Therefore, for the purpose of simply finding a distribution that reasonably reflects the data being analysed, there may effectively be no difference between using a mixture of normal distributions versus a non-normal data distribution (McLachlan and Peel, 2000). However, phenotypes are assumed to be normal due to their dependence on multiple genes in quantitative genetic theory, so it seems a valid assumption in our study that non-normality is likely to be due to the presence of multiple forms. In the present study, it was also already known that this species frequently develops into discrete forms, so fitting mixtures of normal distributions was a natural choice over fitting non-normal distributions. One should refer to more comprehensive sources if fitting mixtures of non-normal distributions is of interest (e.g. Fraley and Raftery, 1998; McLachlan and Peel, 2000; Royle, 2004; Benaglia *et al.*, 2009; Reinecke and Seddig, 2011; Steinly and Brusco, 2011). In addition, although the analyses presented were limited to a single type of data, other software packages or code may be particularly useful if multiple forms of data need to be considered simultaneously (e.g. Webb *et al.*, 2007; Benaglia *et al.*, 2009; Smith and Campana, 2010).

This study has exemplified how diversity below the species level may be quantified using mixture models. As such, intraspecific diversity as resource polymorphism has been

instrumental in the development of ecological speciation theory (Schluter, 1996) and has important effects on both the ecosystem functions (Palkovacs and Post, 2008; Harmon *et al.*, 2009) and rates of evolutionary diversification (Pfennig and McGee, 2010). The application of mixture model methods to aid in comparisons across systems is extremely useful in fields spanning ecology and evolutionary biology; however, the methods presented here are also broadly applicable beyond this purpose. For example, comparisons may be made across time or taxonomic units instead of systems: calculating the number of groups (K) or Differentiation could be used as a temporal indicator to monitor losses in biodiversity. Mixture models can aid management goals by defining a wide variety of biological units, increasing accuracy of parameter estimation, and enabling the placement of intraspecific diversity within a functional context.

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