Genetic and Phenotypic Variation in the Freshwater Crayfish *Pacifastacus leniusculus* (Dana)

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Abstract

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This thesis deals with questions concerning the mechanisms behind genetic and phenotypic variation among Swedish populations of the introduced freshwater crayfish *Pacifastacus leniusculus* (Dana). Discriminant analyses of morphological characters and allozyme electrophoresis were employed to investigate the influence of three subspecies, *P. l. klamathensis*, *P. l. leniusculus* and *P. l. trowbridgii*, on Swedish populations. Morphological and electrophoretic data agreed in separating one of the subspecies, *klamathensis*, from the Swedish populations. It is concluded in the present study, that *P. l. klamathensis* is not represented in Sweden. However, none of the methods could exclude any of the other two subspecies from the Swedish material.

Founder effects in connection with introductions to Sweden, and breeding management, are suggested to be responsible to the absence of rare alleles observed at two loci, and skew allele frequencies observed at one locus. When transplantations within Sweden are concerned, no signs of founder effects were observed. Rare alleles present in the source population were also present in the derivative populations.

In two indoor experiments, I investigated the effects of temperature and food on the maturation of ovaries, and how temperature and egg size affected the development time and the quality of progeny. A slightly decreased temperature during the summer months resulted in spawning of smaller eggs in the autumn. Crayfish feeding on a diet enriched with fishmeat produced eggs that contained larger proportions of lipids. The diet also influenced the fatty acid composition of the eggs. Temperature was inversely correlated to development time of eggs. The size of stage I and stage II juveniles was positively correlated to egg size. Observations of higher proportions of water in small eggs than in large eggs, throughout the development, probably reflect a more rapid development in small eggs. It is suggested that temperature differences among localities to a great extent are responsible to the observed variation in egg size among Swedish populations of *P. leniusculus*.

Anders Agerberg, Institute of Limnology, Uppsala University, S-752 36, Uppsala, Sweden

" There's more to the picture
Than meets the eye "

Neil Young

Preface

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals **I-IV**:

I.   AGERBERG, A. Variation in morphological characters in the freshwater crayfish *Pacifastacus leniusculus* (Dana). - *Manuscript*

II. AGERBERG, A. 1990. Genetic variation in three species of freshwater crayfish; *Astacus astacus* L., *Astacus leptodactylus* Aesch., and *Pacifastacus leniusculus* (Dana), revealed by isozyme electrophoresis. - *Hereditas 113*: 101-108

III. AGERBERG, A. and JANSSON, H. Genetic comparisons between introduced stocks and three subspecies of the freshwater crayfish *Pacifastacus leniusculus* (Dana), based on allozyme data.- *Manuscript*

IV. AGERBERG, A. Effects of temperature and egg size on embryonic development in the freshwater crayfish *Pacifastacus leniusculus* (Dana). - *Manuscript*

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Contents

Introduction

Variability among individuals may be attributed to factors such as individual genetic variation, difference in age, sex, food, stage of life cycle, and habitat. Falconer (1989) introduced the concept *value*, for which a character is measured. Observations of characters such as morphology, physiology, or behaviour of an organism, constitute the phenotypic value of that individual. The phenotypic value is divided into components which can be ascribed to the influence of the genotype, the environment, and their interaction. The genotype is the particular assemblage of genes possessed by the individual, and the environment is all the non-genetic circumstances that influence the phenotypic value. As each genotype is able to respond within its reaction norm to changes in the environment, the degree of this phenotypic plasticity may differ among individuals.

The genotypic composition of a population may change by migration, genetic drift, and natural selection. The founder effect, caused by few individuals involved in the establishment of new colonies, forms a special case of genetic drift. The consequence on the genotypic composition for a population passing through founder events, is decreased genetic variation which may be expressed as (*i*) loss of alleles relative to the source population (Chakraborty and Nei 1977, Leary et al. 1985, Maruyama and Fuerst 1985, Singh 1990) or (*ii*) decreased heterozygosity due to inbreeding in the following generations (Ståhl 1983, Vuorinen 1984, Leary et al. 1985, Hedrick 1990). The rate of inbreeding is expected to be high in species with overlapping generations (Hill 1979). Loss of genetic variation has been shown to have negative effects on fitness-related characters (see Kincaid 1983 for review).

Populations which are reproductively isolated from each other, are assumed to begin to diverge genetically because of genetic drift and selection (Mayr 1963). From a number of electrophoretic investigations at intra- and interspecific level there seems to be a low degree of genetic differentiation associated with the early stages of speciation. In a review of statistics, for the lower levels of evolutionary divergence, Singh (1990) found that local populations displayed genetic identity of more than 97%, subspecies between 80-90%, and sibling species between 46-78%. It is obvious that distinct sub-species on the average show much greater genetic differentiation than populations which have not been deemed to merit subspecies status. However, in many cases the molecular genetic results have contradicted previous ideas about species integrity or taxonomic distinctions, that were based on morphological descriptions (Turner and Grosse 1980, Busack and Gall 1981, Phelps and Allendorf 1984).

To solve disputes on species versus subspecies status, both extensive morphological data and molecular genetic analyses should be applied (O'Brien

and Mayr 1991). In a modification of the former criteria for subspecies (Mayr 1940, 1970), O'Brien and Mayr (1991) stated that "members of a subspecies share a unique geographic range or habitat, a group of phylogenetically concordant phenotypic characters, and a unique natural history relative to other subdivisions of the species".

The growing interest in aquaculture has led to extensive cultivation, stocking, and restocking of a number of species, among them also freshwater crayfish. This type of activities include management programs to produce crayfish for release into natural waters. However, transplantations of native and exotic crayfish are mostly conducted without the neccesary knowledge about the genetic characteristics of the stocking material. One central problem involved in all transplantations of organisms into natural localities or hatcheries, concerns the number of individuals used. The use of a small number of founders, increases the risk of inbreeding and may lead to unwanted genetical and phenotypical changes (Falconer 1989). In the Swedish populations of *P. leniusculus*, founder effects may have occurred in connection with introductions from North America or in connection with subsequent transplantations within Sweden. The introduction of *P. leniusculus* to Sweden may have involved the mixing of subspecies. Three subspecies, *P. l. klamathensis*, *P. l. leniusculus* and *P. l. trowbridgii*, occur in those areas in California, from which the founder animals of two Swedish source populations have been collected. The third source population originates from British Columbia where the two last-mentioned subspecies occur (Hobbs 1974).

Populations genetically differentiated due to natural selection or genetic drift may exhibit differences in reproductive parameters, such as fecundity and egg size. Variation in reproductive characteristics in crustaceans, such as egg weight, is suggested to be determined by a combination of genetic and non-genetic forces (Munro and White 1975, Botrell et al. 1976, Lonsdale and Levinton 1985). Considerable intraspecific variability in egg size has been demonstrated in crustaceans (Clarke 1979, Van Dolah and Bird 1980, Lonsdale and Levinton 1985). There are several reasons to suspect that the Swedish populations of *P. leniusculus* express variations in reproductive characteristics. 1) The introductions have been carried out from different localities in the North America. Different reproductive strategies may have evolved among the ancestor populations due to variation in the environment. 2) Founder effects in connection with the introduction to Sweden may have changed the genotypic composition, thus influencing the reproduction. 3) It can not be excluded that different subspecies have influenced the Swedish material. 4) Differences in the environment among the localities, such as temperature and food availability may cause physiological effects on the reproduction.

The present study is an analysis of genotypic and phenotypic variation

within one species, the freshwater crayfish *Pacifastacus leniusculus* (Dana). The objectives of the study were to investigate: (*i*) the relative influence of three subspecies within *P. leniusculus* on the populations introduced to Sweden from different ancestor localities in North America. (*ii*) if subsequent transplantations within Sweden have resulted in any structural genetic changes in the derivative populations relative to their Swedish source populations. (*iii*) the influence of temperature and food on the developing ova, as regards size and quality of the oviposited eggs. (*iv*) the effect of temperature on the egg development time, and the ecological consequences of egg size on size and quality of the hatchlings.

The species

P. leniusculus is native to the western parts of North America (Miller 1960). The species contains three subspecies: *Pacifastacus leniusculus klamathensis* (Stimpson), *Pacifastacus leniusculus leniusculus* (Dana) and *Pacifastacus leniusculus trowbridgii* (Stimpson). The subspecific status is valid according to a taxonomic revision by Miller (1960), based on morphological and zoogeographical data. Prior to the revision, the subspecies were claimed to be separate species. However, the systematics regarding *P. leniusculus* has been a matter of dispute. Frequent observations of crayfish, morphologically intergrading between *P. l. leniusculus* and *P. l. trowbridgii*, indicated an extensive intraspecific phenotypic plasticity, according to Riegel (1959). Miller (1960), on the other hand, suggested that the intermediate forms were intergrade progeny from original introductions. This suggestion was based on observations of forms being morphologically intermediate between the subspecies along contact zones, whereas the subspecies were morphologically distinct from each other at isolated localities.

    P. l. klamathensis is the only subspecies native to California (Miller 1960, McGriff unpubl.), but subsequent introductions of crayfish to localities in California around the turn of the century (Faxon 1914 cited in Miller 1960, Riegel 1959, Abrahamsson and Goldman 1970) have resulted in the occurrence of all three subspecies and hybrids between the subspecies.

    Both *P. l. leniusculus* and *P. l. trowbridgii* are indigenous to the Columbia River System in Oregon and Washington. According to Miller (1960), *P. l. leniusculus* occupies deep rivers and sloughs, whereas *P. l. trowbridgii* is found in more varied environments. The two subspecies are sometimes reported to share niches and to intermingle during migration movements in connection with reproduction in the autumn. The morphological discrimination between the subspecies is principally based on shape of chelae and spinal characteristics
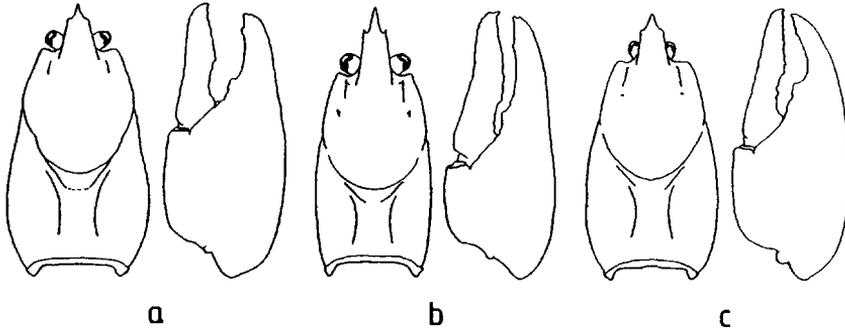
FIGURE 1. Dorsal view of carapace and chelae of males of: **a)** *P. l. klamathensis*, **b)** *P. l. leniusculus*, and **c)** *P. l. trowbridgii*. Redrawn after Hobbs (1974).

(Miller 1960, Hobbs 1974) (Fig. 1). The three subspecies differ in maximum size, with *P. l. leniusculus* being the largest, *P. l. klamathensis* the smallest and *P. l. trowbridgii* intermediate.

Since 1907 the crayfish plague, caused by the fungus *Aphanomyces astaci* Schikora, has devastated a large number of Swedish populations of the native crayfish *Astacus astacus* L.. During the 1950s investigations were initialized to find an exotic crayfish species which could resist the plague and establish vigorous populations in Sweden. A survey of 16 American species finally resulted in that *Pacifastacus leniusculus* (Dana) was introduced (Svärdson 1959). This species, also known as the signal crayfish, was reported to be equivalent to *A. astacus* regarding reproduction and growth, and was furthermore resistant to the crayfish plague (Svärdson 1965).

The first introduction was carried out in 1960, when a limited number of crayfish were shipped from the American River, California, and introduced to a small Swedish lake, Träsksjön, close to Stockholm. Additional introductions from other areas have been made afterwards. During the years 1969-1976 a large number of crayfish (>50 000) from Donner Lake and Lake Tahoe were imported to Simontorp Aquaculture AB. A third introduction was made in 1975 to a small pond in Kvarntorp some 180 km west of Stockholm. This sample, originating from Pitt Lake, British Columbia, Canada, constituted approximatively 200 individuals. In connection with the introduction, the sample from Pitt Lake was subject to breeding management during some years, thus the actual number of introduced individuals is unclear (B. Ideström pers. comm.). Since the initial

A 1 Träsksjön
A 2 Erken
B 1 Simontorp
B 2 Halmsjön
B 3 Skillötsjön
C 1 Kvarntorp

Pitt Lake, British Columbia
1975 $N = 200$

American River, California
1960 $N = 56$

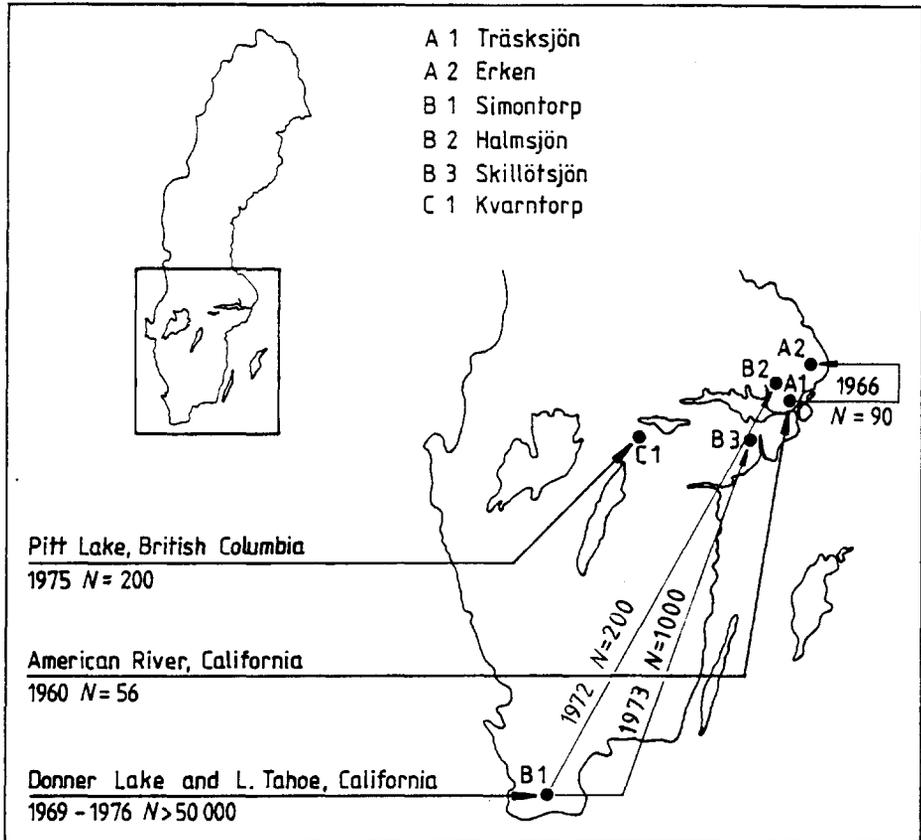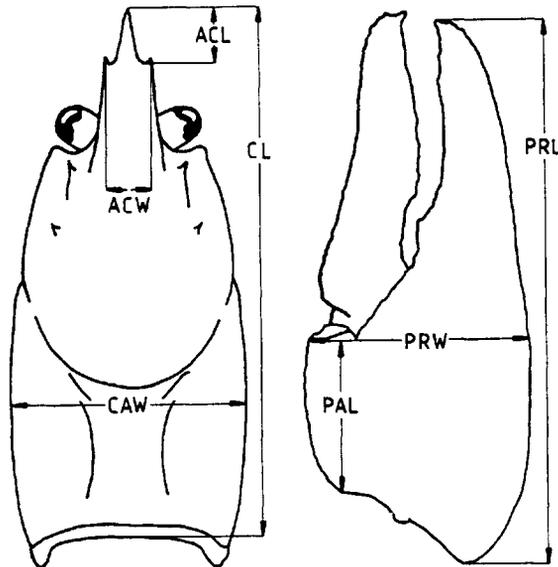Donner Lake and L. Tahoe, California
1969 – 1976 $N > 50\,000$

FIGURE 2. Origin of the Swedish populations of *P. leniusculus* investigated in the present study.

introductions subsequent transplantations have been carried out within Sweden (Svärdson et al. 1991) (Fig. 2) and to a number of other countries in Europe (Lowery and Holdich 1988).

Analytical methods

Multivariate discriminant analysis of morphometric characters (I)

Six morphological characters were applied to the discriminant analyses (Fig. 3): Length of acumen from base of lateral spines (ACL), width of acumen at base of lateral spines (ACW), width of carapace (CAW), length of palm (PAL), length of propodus (PRL), width of propodus at base of dactylus (PRW). Length of carapace (CL) was used as the standard size measurement. All measurements were made to the nearest 0.01 mm with dial calipers. Sexual dimorphism required the sexes to be analysed separately. All measurements were adjusted to those expected for mean body size by an allometric formula (Thorpe 1975).

FIGURE 3. Measurement recorded: Acumen length (ACL), acumen width (ACW), carapace width (CAW), carapace length (PAL), propodus length (PRL), and propodus width (PRW). Carapace length is used as a standard size measurement.

The discriminant analyses were conducted in two separate runs. In the first run the discriminant functions were obtained from the three subspecies, sampled from localities in the United States. Thereafter the Swedish source populations were entered to the calibration data set. In the second run, the Swedish source populations constituted the calibration data set on which the Swedish derivative populations were tested. The canonical discriminant analyses were carried out in order to investigate the relative influence of the morphological variables on the discrimination. Prior to the discriminant analyses, the Shapiro-Wilk statistics were computed to test for multivariate normality.

Electrophoretic analyses (II, III)

Isozyme electrophoresis was used to study the genetic variation within *P. leniusculus*. All electrophoretic analyses were carried out on frozen material. From each individual, extracts from five different tissues were taken out. Prior to analysis, samples of tissues were homogenized in a phosphate-sucrose grinding solution and centrifuged at 10 000 rpm. Horizontal gel electrophoresis was performed in 11% starch gels. The buffer systems and the staining procedures used are described in papers **II** and **III**.

Biochemical composition of eggs and offspring (IV)

Carbon and nitrogen content was measured in lyophilized material and in ash from eggs, stage I progeny, and stage II progeny. Ash was obtained after ignition in a muffle furnace at 450 °C for 12 hours. Carbon and nitrogen was measured with a Carlo Erba 1106 elemental analyser. As a standard, acetanilide (carbon 71.08% and nitrogen 10.36%) was used. Combustion temperature was 1025°C. Prior to combustion, the samples were weighed on a Cahn electro-microbalance and sealed in tin capsules. Proximate biochemical composition (carbohydrate, lipid and protein) was determined according to Gnaiger and Bitterlich (1984). The fatty acid composition of eggs and offspring was estimated on chloroform-methanol extracts of lyophilized material with a Hewlett Packard 5890 GLC system, using a Nordion fused silica capillary column, NS-351, 25 m (Boberg et al. 1985). As internal standard 0.25 mg of the fatty acid 22:0 was used.

Results and discussion

Relative influence of the three subspecies on the Swedish populations (I, II, III)

The discriminant analyses aimed at developing a classification data set, comprising the three subspecies. For both sexes, the analysis revealed a good discrimination between the subspecies as the percentage of misclassification never exceeded 10% (I). When the Swedish source populations, Träsksjön, Simontorp, and Halmsjön, were compared to the three subspecies, no single individual was classified as *P. l. klamathensis* (Table 1). All male individuals showed a closer resemblance to *P. l. trowbridgii* (>68%) than to *P. l. leniusculus* (<32%). However, females from Simontorp and to a certain extent from Träsksjön, were classified as *P. l. leniusculus* (>48%). The reason for this divergence in classification between sexes is probably found in the weaker discrimination between females than males of the two subspecies. Males of the two subspecies expressed pronounced differences regarding palm length (PAL). Females, however, mainly differed in traits associated with shape of acumen, characters which expressed a large amount of heterogeneity within populations.

The development of an electrophoretic technique (II) initially comprised 21 enzymes and non-specific proteins. *P. leniusculus* exhibited the highest degree of polymorphism (three loci) of three species included (besides *P. leniusculus* also *A. astacus* and *A. leptodactylus* were included). The variable loci were observed in the enzyme systems diaphorase (*DIA-2**), malate dehydrogenase (*MDH-1**), and phosphoglucomutase (*PGM**). The observation of three polymorphic loci out of 21 (if general proteins are omitted) in *P. leniusculus* is low (14%), compared to the 35% which is the average estimate for animal species in general (see Ferguson 1980 for review). However, a low degree of genetic variation seems to be a general attribute of freshwater crayfish (Brown 1981, Busack 1988, 1989) as well as of decapod crustaceans (Nelson and Hedgecock 1980). In one locus of esterase, variation was observed which could be characterized as being of non-genetic origin. It was suggested that the divergent allele expression at this locus was due to differences in the treatment of the animals. Non-genetic variation in esterase has also been reported in a previous study of crayfish (Fevolden and Hessen 1989).

TABLE 1. Summary of classifications by quadratic discriminant function of *P. leniusculus* showing percent of observations classified into subspecies. Asterisks indicate classifications in accordance to a priori information.

Subspecies/ population	Sex	*N*	Classified into		
			P. l. klamathensis	*P. l. leniusculus*	*P. l. trowbridgii*
P. l. klamathensis	f	18	100.0*	0.0	0.0
	m	23	100.0*	0.0	0.0
P. l. leniusculus	f	61	0.0	95.1*	4.9
	m	46	0.0	97.8*	2.2
P. l. trowbridgii	f	26	0.0	3.9	96.2*
	m	21	0.0	9.5	90.5*
Kvarntorp	f	33	0.0	27.3	72.7
	m	25	0.0	32.0	68.0
Simontorp	f	36	0.0	63.9	36.1
	m	20	0.0	25.0	75.0
Träsksjön	f	27	0.0	48.2	51.9
	m	26	0.0	23.1	76.9

The technique described above was applied to the three subspecies and to the Swedish populations (**III**). Polymorphism could be detected in four loci, *GPI** (*P. l. leniusculus* only), *IDHP-2** (*P. l. klamathensis* only), *MDH-1** (*P. l. leniusculus*, *P. l. trowbridgii* and the Swedish populations), and *PGM** (*P. l. leniusculus* , *P. l. trowbridgii* and one Swedish source population) (Table 2). One locus, *DIA-2** , that had previously been reported as polymorphic (**II**), was excluded due to weak activity. The taxonomical separation into subspecies was justified by contingency chi-square analysis of allele frequencies (p<0.001). However, the separation between *P. l. leniusculus* and *P. l. trowbridgii* was less pronounced when average genetic identity (Nei 1978) was estimated at polymorphic loci among pairs of subspecies (I=0.985 between *P. l. leniusculus* and *P. l. trowbridgii*, but only 0.757 ± 0.028 between *P. l. klamathensis* and the other two subspecies). When the Swedish source populations were compared to the three subspecies (Fig. 4 and Table 3), *P. l. klamathensis* appeared to be separated from all Swedish populations (average genetic identity, I=0.711). However, it was not possible to discriminate any of the Swedish populations either from *P. l. leniusculus* or from *P. l. trowbridgii*. Estimates of average genetic identity between the Swedish populations and these two subspecies were close to 1.0 (0.986 and 0.966, respectively). When observations of present/absent alleles are concerned, all alleles observed in *P. l. leniusculus* and *P. l. trowbridgii* were also expressed in at least one of the Swedish source populations, with the exception of rare alleles in *GPI** and *PGM**, which only were observed in a single specimen of *P. l. leniusculus* from the American material.

Morphological and electrophoretic data are concordant regarding the systematics of subspecies as well as the character of the Swedish populations of *P. leniusculus*. Consequently it can be suggested that one subspecies, *P. l. klamathensis*, has not influenced the Swedish populations. When considering the other two subspecies, neither morphological nor electrophoretic analyses could separate them from Swedish populations. In spite of mutual separation regarding allele frequency homogeneity and morphological characters, *P. l. leniusculus* and *P. l. trowbridgii* are considerably more identical to each other than to *P. l. klamathensis* in both genetical and morphological respects.
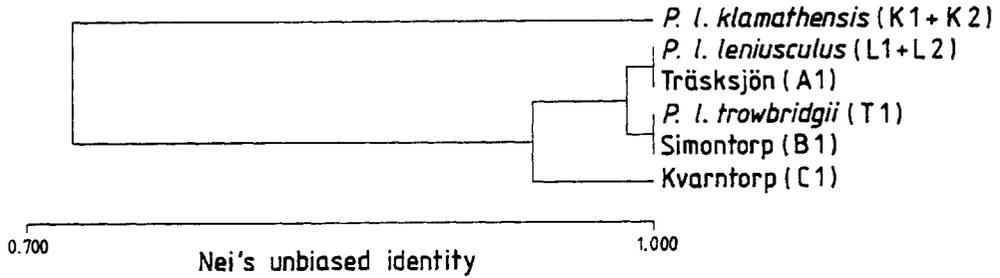
TABLE 2. Allele frequencies at polymorphic loci and observed and expected average heterozygosity at polymorphic loci. The subspecies are designated K1 and K2 (*P. l. klamathensis*), L1 and L2 (*P. l. leniusculus*), and T1 (*P. l. trowbridgii*). When designation of introduced population is considered, see Fig. 2.

Locus	Allele	Locality designation										
		Subspecies					Introduced populations					
		K1	K2	L1	L2	T1	A1	A2	B1	B2	B3	C1
*GPI**	*90	–	–	–	0.022	–	–	–	–	–	–	–
	*100	1.000	1.000	1.000	0.978	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	N	48	49	41	45	45	40	48	40	55	39	36
*IDHP-2**	*90	0.926	0.784	–	–	–	–	–	–	–	–	–
	*100	0.074	0.216	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	N	47	44	44	46	44	40	55	40	55	40	36
*MDH-1**	*70	–	–	–	–	–	–	–	0.041	0.066	0.029	–
	*100	1.000	1.000	0.500	0.565	0.760	0.520	0.500	0.680	0.472	0.514	0.194
	*125	–	–	0.500	0.435	0.240	0.480	0.500	0.279	0.462	0.457	0.906
	N	54	50	56	54	52	51	50	61	53	69	36
*PGM**	*95	–	–	–	0.011	–	–	–	–	–	–	–
	*100	1.000	1.000	0.887	0.913	0.977	1.000	1.000	0.917	0.940	0.905	1.000
	*105	–	–	0.112	0.076	0.023	–	–	0.083	0.060	0.095	–
	N	51	46	40	46	43	47	55	48	50	42	34
H_P obs.		0.037	0.074	0.190	0.169	0.072	0.103	0.120	0.140	0.157	0.174	0.083
H_P exp.		0.035	0.086	0.177	0.176	0.104	0.126	0.126	0.154	0.170	0.176	0.079

H_P=Average heterozygosity at polymorphoc loci.
exp.=Nei (1978) unbiased estimate.

This makes the subspecies status of the two former taxa uncertain. Compared to levels of genetic identity obtained from a variety of organisms (see Singh 1990 for review), the identity between *P. l. leniusculus* and *P. l. trowbridgii* in this study is on a level that would be expected for local populations.

FIGURE 4. Relative genetic identity dendrogram generated by UPGMA (Sneath and Sokal 1973) of Nei's (1978) unbiased genetic identity coefficients, calculated among three subspecies and Swedish source populations of *P. leniusculus*, using four polymorphic loci. Within brackets are population designations.

TABLE 3. Pairwise comparisons of genetic identity and distance at polymorphic loci among subspecies and Swedish source populations of P. leniusculus. Above: Nei's (1978) unbiased identity. Below: Nei's (1978) unbiased distance.

Subspecies/population	(K1+K2)	(L1+L2)	(T1)	(A1)	(B1)	(C1)
P. l. klamathensis (K1+K2)	–	0.729	0.785	0.735	0.769	0.629
P. l. leniusculus (L1+L2)	0.316	–	0.985	0.999	0.992	0.967
P. l. trowbridgii (T1)	0.242	0.015	–	0.985	0.999	0.913
Träsksjön (A1)	0.308	0.001	0.015	–	0.990	0.972
Simontorp (B1)	0.263	0.008	0.001	0.010	–	0.972
Kvarntorp (C1)	0.464	0.033	0.091	0.028	0.075	–

Phenotypic and genotypic changes related to founder events (I, III)

Individuals from the derivative populations could to a great extent (averaging 60%, when males from Erken are omitted) be classified into their respective source locality (I). However, male crayfish from Erken exhibited a correct classification of only 27.8%. This was probably due to deviation in one morphological character, propodus width (PRW), between male crayfish from Erken and its source locality Träsksjön. In the scattergram obtained from canonical discriminant analysis of males (Fig. 5), the derivative populations are placed close to their respective source populations along the most discriminating variable (CAN1). This variable mainly reflects variation in palm length (PAL). Along the second variable (CAN2), which is almost entirely attributed to variation in propodus width, there is a separation between Erken and Träsksjön. Thus, the conclusion that the palm length is a very useful taxonomic character when separating subspecies of *P. leniusculus*, also comprising identification at the population level.

No genetic genetic evidence was found of founder effects, such as loss of alleles or reduced heterozygosity, in comparisons between derivative populations and their respective source populations (III). If rare alleles were present in the source population, they were also observed in the derivative population (Simontorp, Halmsjön and Skillötsjön). Likewise, lack of rare alleles was followed by a parallel lack in its derivative population. Furthermore, we found no evidence of inbreeding, such as decreased heterozygosity, in the derivative populations relative to their Swedish source population.

The reasons for differences regarding present/absent alleles in the source populations may only be speculative, as no data exist from the ancestor localities in North America. Two source populations, Träsksjön and Kvarntorp, showed an absence of rare alleles that were observed in the Simontorp population at two loci (*MDH-1** and *PGM**) (Table 2). The alleles occurred in low frequencies, averaging 4.5% and 7.9% respectively. The introduction to Träsksjön contained only a few individuals ($N=56$). The Kvarntorp population was based on approximately 200 crayfish being shipped from British Columbia. This number was most likely further reduced due to breeding management prior to introduction into natural waters (B. Ideström pers. comm.). In none of the introductions the sex ratio is known. Any shift in ratio from 1:1 would further reduce the effective population size (Falconer 1989). Simple calculations of changes of allele frequencies after a founder effect can be carried out, assuming that the frequencies observed in Simontorp and its derivative localities were applicable to all ancestral localities. From these calculations it can be suggested that the absence of rare alleles in the Kvarntorp and Träsksjön populations is the result of founder effects in connection with the respective introductions.
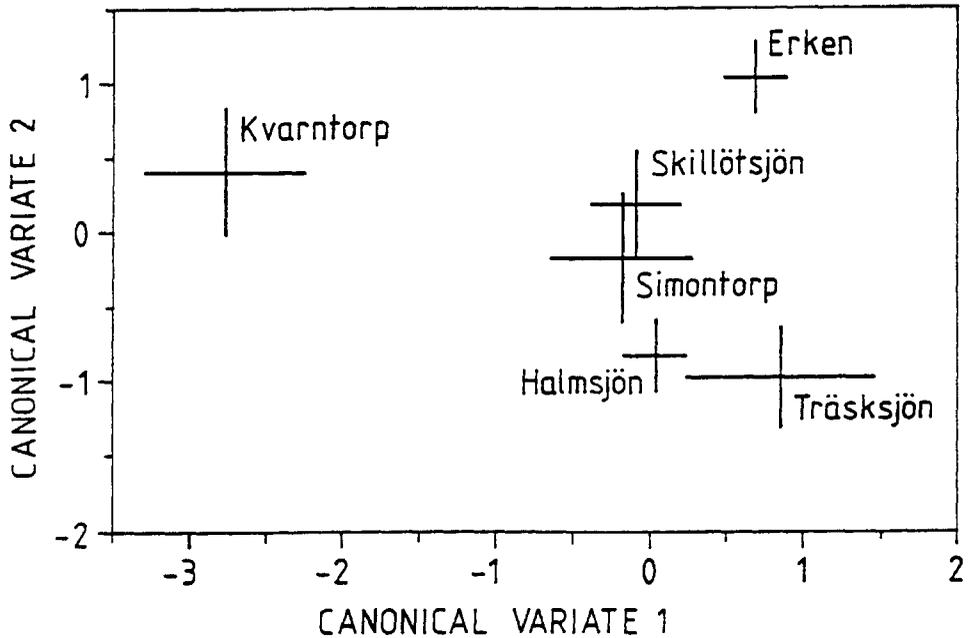
FIGURE 5. Distribution of mean scores and 95% confidence limits for group centroids at the first two canonical variables of Swedish source populations and derivative populations of male *P. leniusculus*.

Observation of skewed allele frequencies in the Kvarntorp population at *MDH-1**, relative to the other populations, strengthens the conclusion regarding founder effect in this population. However, it must be emphasized that the conclusion is based on the assumption that rare alleles are expressed in the ancestral localities. The certainty of this assumption may be questioned, as one of the rare alleles (*MDH-1* 70*) was not detected in any of the subspecies populations.

Non-genetic factors influencing the embryonic development, and the ecological significance of egg size (IV)

Differences in dry weight of newly spawned eggs occured among populations of *P. leniusculus* from different lakes. These differences can either be attributed to genetic differentiation or to varying environmental conditions between the localities. To evaluate the effects of two environmental factors, food quality and temperature, on the maturation of ova, a laboratory experiment was set up. In the experiment, which extended from June until spawning, which occurred in October, females were exposed to different diets and temperatures. The two diets differed with respect to protein and lipid content. Female crayfish served food with a supplement of fish (rich diet) generally produced eggs that contained a larger proportion of lipids than females without this extra supplement. Significant differences were obtained between eggs reared at low temperature, but not at elevated temperature (Wilcoxon Rank Sum test, $P<0.05$ and $P>0.07$, respectively). The composition of fatty acids in the eggs differed between food treatments. Eggs from the group fed on a poor diet exhibited a larger proportion of essential fatty acids and correspondingly a smaller proportion of monounsaturated fatty acids. This indicates that essential fatty acids are preserved and not consumed in conditions of low food availability. When given a rich diet, females exposed to elevated temperature produced eggs with higher dry weight than females exposed to reduced temperature (Wilcoxon Rank Sum test, $P<0.001$). No significant differences were obtained between temperature treatments when the diet was poor ($P>0.37$). This indicates the presence of an interactive influence of temperature and food on the maturation process.

In an indoor experiment the eggs were stripped from ovigerous females sampled from three populations, and the embryonic development was studied. The experiment lasted from May until hatching in the summer, at three different temperature regimes. An inverse relationship existed between embryonic duration and temperature. The number of degree days required for the eggs to hatch significantly differed between the localities (Kruskal-Wallis test, $P<0.05$). Varying environmental conditions among the localities is suggested to be responsible for the observed variation in development time.

During the development the water content in the eggs increased. A higher water content in the small eggs is suggested to reflect a faster development relative to large eggs. Lipid was the main energy source for the eggs during development. The proportions of essential fatty acids ($\omega3$ and $\omega6$) increased throughout the development. This is consistent with the results from the study of ovarian development (see above), and shows that polyunsaturated fatty acids to a great extent are omitted from energy utilization. There was no difference in lipid content between eggs from different size classes ($P>0.15$). However, there
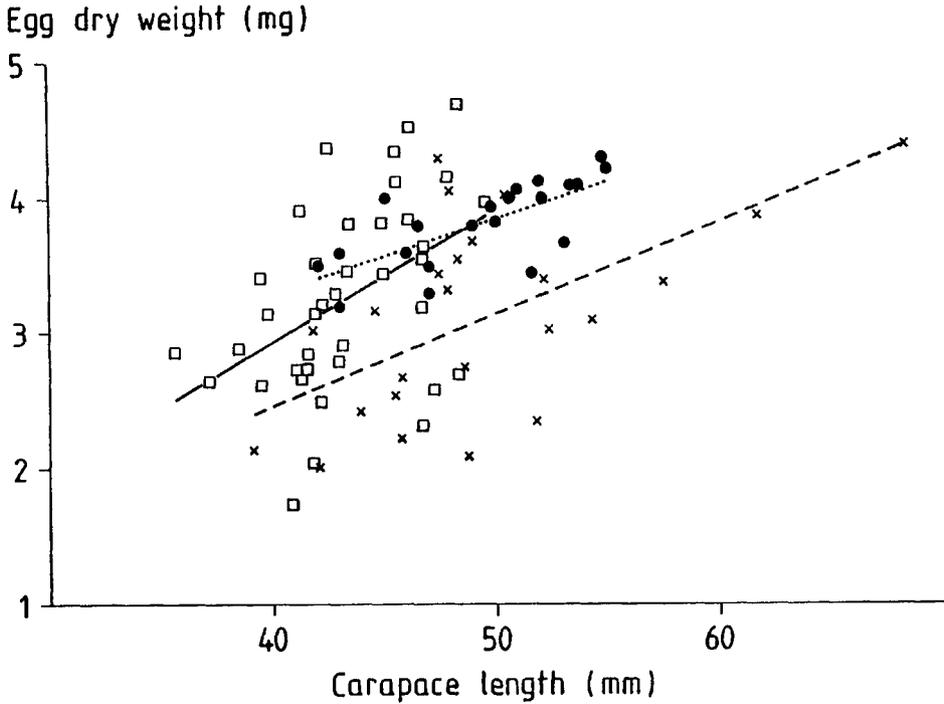
FIGURE 6. Dry weight of oviposited eggs, and carapace size of female *P. leniusculus*, obtained from three Swedish localities: Halmsjön (squares and continuous line, n=38), Erken (crosses and hatched line, n=25), and Kvarntorp (filled circles and dotted line, n=21).

was a significant positive relationship between dry weight of eggs and dry weights of stage I and stage II juveniles (Kruskal-Wallis test, $P<0.01$ and $P<0.05$, respectively) (Fig. 7).

It can thus be concluded that temperature has a strong effect on the embryonic development with respect to the size of oviposited eggs and rate of development. It may therefore be suggested that egg size variation among localities to a great extent is caused by differences in temperature. However, genetic influences can not be ruled out. The ecological significance of producing large eggs is that larger and more competitive progeny will be the result. The study also showed the importance of food quality on lipid content of the eggs. Lipids constituted the main energy source in the eggs during the embryonic development.
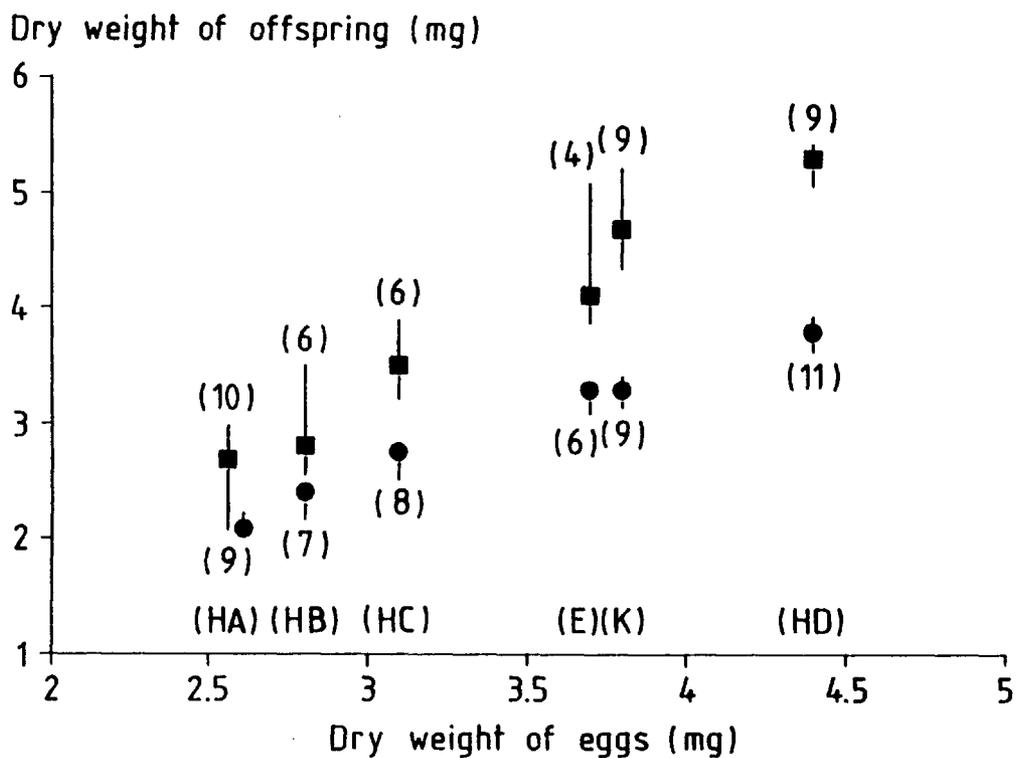
FIGURE 7. Dry weights of stage I juveniles (filled circles), and stage II juveniles (filled squares), related to dry weights of eggs. Sample designations are showed within brackets (HA-HD = Halmsjön A-D, E = Erken, and K = Kvarntorp).

Conclusions

Based on the American material, both morphological and electrophoretic methods revealed a pronounced separation of *P. l. klamathensis* from the other two subspecies, *P. l. leniusculus* and *P. l. trowbridgii*. It may also be concluded from the present study, that none of the stocks introduced to Sweden contained the subspecies *P. l. klamathensis*.

No loss of alleles was detected in comparisons between derivative populations and their respective source population. Thus, no indications of founder effects in connection with transplantations within Sweden were found. However, differences between the Swedish source populations as regards presence/absence of rare alleles and skew allele frequencies may be attributed to founder effects in connection with the primary introduction.

Food quality during the maturation of ovaries influenced the lipid content and the composition of fatty acids in the eggs at oviposition. Temperature was found to be an important factor regulating the maturation of ova and the development time to hatching of eggs. Thus, variable temperature conditions are suggested to be the main cause to variation in egg size among Swedish populations of *P. leniusculus*. However, a genetically induced variation can not be excluded. Different reproductive strategies among the ancestor populations, may have evolved due to varying environmental pressure. It is concluded that the production of large eggs is attained at the expense of fecundity and hatching time. On the other hand, the advantage is the production of larger and consequently more competitive progeny.

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