Ann. Zool. Fennici 35: 21–27
Helsinki 4 June 1998
ISSN 0003-455X

Do three-spined sticklebacks group with kin?

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Received 3 October 1997, accepted 11 December 1997

We studied the kin structure in schools of juvenile three-spined sticklebacks (*Gasterosteus aculeatus*) using allozymes as genetic markers. Young of the three-spined stickleback stay in their natal nest for several days after hatching. They thus have an opportunity to associate with kin when leaving the nest for independent life. Juveniles have also been shown to preferentially associate with kin in laboratory conditions, and furthermore, schools of free-ranging juveniles are size-assorted, all suggesting that schools might consist of relatives in the wild. However, our data show no close relatedness among individuals within the schools, indicating that school members are a random sample from the genetic pool of the population. The result therefore suggests that, despite young sticklebacks' apparent opportunity to group with kin, their school formation in the wild is not tightly linked with genetic relatedness.

1. Introduction

There are various costs and benefits of group life potentially affecting individual fitness (Alexander 1974, Bertram 1978, Clark & Mangel 1984, Pulliam & Caraco 1984, Magurran 1990, Pitcher & Parrish 1993). The direct costs and benefits may not be shared equally among the group members, some individuals benefiting more while others being prone to pay more costs, thus faring less well in direct fitness terms (Pusey & Packer 1997). For example, in groups of fish the behaviour of some individuals, such as predator inspection, may increase their mortality risk (e.g., Milinski *et al.* 1997). The others staying behind may still share the benefits of inspection with less costs (Pitcher & Parrish 1993). According to Hamilton's rule, selection favours animals that perform acts for which

$$r \times b - c > 0 \qquad (1)$$

where r stands for relatedness, b is the benefit received by the recipient of an act (ultimately in terms of increased direct reproduction), and c is the cost paid by the actor (in terms of decreased direct reproduction) (Hamilton 1964). Accordingly, in social groups consisting of kin, costly acts, in terms of an individual's direct fitness, may yet be favoured through kin selection.

There is evidence from a number of fish spe-

cies that individuals can discriminate kin (e.g., Quinn & Busack 1985, Olsén 1989, FitzGerald & Morrissette 1992, Brown & Brown 1993, Brown *et al.* 1993, Olsén *et al.* 1996) and often prefer to associate with them (e.g., Olsén 1989, Quinn & Hara 1986, FitzGerald & Morrissette 1992). Still, only a few studies have considered the genetic structure in groups of fish — the most common interpretation being that individuals do not associate with relatives (Avise & Shapiro 1986, Dowling & Moore 1986, Naish *et al.* 1993, but *see* Ferguson & Noakes 1981). But, owing to varying life-history traits among different fish species (Wootton 1991), it is possible that individuals of some species have better opportunities to associate with their kin than others. Parental care, for example, allows closely related young fish to remain together during the guarding phase. An opportunity for kin-group formation thus arises and may persist also through later stages. However, no studies investigating the genetic structure of schools in a species with parental care are available.

Here we studied whether juvenile schools of the three-spined stickleback (*Gasterosteus aculeatus*) consist of kin originating from the same nest. In the three-spined stickleback, the male cares for eggs and fry during the first days after their hatching (Whoriskey & FitzGerald 1994). Since the number of parents contributing to the progeny of each nest seems relatively low (Rico *et al.* 1991, 1992), the young in the nest are expected to be closely related (see below). If the young stay together after they leave the guarding male for independent life, also members of the juvenile schools are expected to be related. Two lines of evidence support this expectation. First, three-spined stickleback young appear to preferentially associate with their kin in laboratory conditions (Van Havre & FitzGerald 1988, FitzGerald & Morrissette 1992), and may do the same also in the wild. Second, schools of juvenile three-spined sticklebacks are size-assorted in the wild (Peuhkuri *et al.* 1997). Although differences in foraging ability and/or in predation risk could account for size-assortative schooling (e.g., Ranta *et al.* 1994), the similarity in individual body size within schools could also result from juvenile schools being groups of kin from the same nest.

2. Material and methods

2.1. Sampling

We captured with a sweep net 24 schools of three-spined stickleback juveniles from shallow littoral areas of the Baltic Sea near Tvärminne Zoological Station, Southern Finland. When collecting the schools, we made sure that only clearly separate schools were caught. The number of individuals within the schools ranged from six to 50. The fish were stored in – 80°C until the allozyme analysis.

2.2. Electrophoresis

We analysed either all individuals or, in the largest schools, up to 30 individuals with horizontal starch gel electrophoresis. In total, 397 individuals were analysed, an average number from each group being 15 (range: 6–30). We screened a total of 23 enzyme systems using standard staining methods (Harris & Hopkinson 1976). Fifteen loci could be reliably scored. Of these, eight were polymorphic, and were used to estimate genetic parameters (*see* Table 1 for the loci studied and buffers used).

2.3. Kin structure of the schools

Genetic relatedness is a standard method for describing genetic colony structure of haplo-diploid social insects (e.g., Crozier & Pamilo 1996), but it is perfectly applicable for group-living diploid organisms as well (e.g., Avise & Shapiro 1986, Costa & Ross 1993). Relatedness can be defined as the genotypic similarity among interactants in a local environment using the population as a reference (Pamilo & Crozier 1982, Pamilo 1989). The local environment in our case is the social group (fish school), and the interactants are members of this group.

We estimated relatedness (r) from the genotype frequency data following Queller & Goodnight (1989) using software Relatedness 4.2 (Goodnight & Queller 1994). We also estimated the mating structure of the population (panmixis) using the

inbreeding coefficient F, which estimates the deviation of the observed heterozygosity (H_O) from the expected one (H_E) according to the Hardy-Weinberg formula:

$$F = (1 - H_O / H_E) \quad (2)$$

(Wright 1951). Standard errors for single locus estimates were obtained by jackknifing over groups, and those for population estimates were obtained by jackknifing over groups and loci (Efron & Tibshirani 1993). For the relatedness estimates the standard errors were used to calculate 95% confidence interval of the mean. Confidence interval was used to make statistical inference on the relatedness within the groups, i.e., whether relatedness deviates from the expected value, $r = 0.25$ (*see* below), as well as whether it deviates from zero indicating no relatedness at all. With inbreeding estimates the standard errors were used to test with t-test the deviation of the obtained values from the expected one, $F = 0$ (total panmixis).

When groups of diploid organisms belong to the same generation, and mating in the population is random, the genetic relatedness within the group (r) is determined by the number of females (P) and males (M) contributing to this group, and relatedness among them (rP and rM, respectively):

$$r = \frac{0.25}{P} + \frac{rP}{4} \times \frac{P-1}{P} + \frac{0.25}{M} + \frac{rM}{4} \times \frac{M-1}{M} \quad (3)$$

(Queller 1993). The numbers of females and males here refer to genetically effective numbers, which assume equal reproduction by each mother and father.

In the three-spined stickleback, the nest owner fathers a majority of the young, although some eggs may be fertilized by sneaker males (Goldschmidt *et al.* 1992, Rico *et al.* 1992). Several females may lay eggs in a single nest (e.g., Kedney *et al.* 1987). However, the genetic data available revealed that usually not more than two females mothered the offspring in a single nest, and, even more importantly, the contribution of the second (or third) female was very low (Rico *et al.* 1991, 1992). We can thus assume that the effective number of both females and males contributing to each nest is two. Furthermore, we can assume the parents to be unrelated because the population is panmictic (*see* Results). If we substitute $P = M = 2$ and $rP = rM = 0$ in Eq. 3, the expected relatedness among the progeny of a single nest is $r = 0.25$. So, if the young associated with their nest mates when leaving the guarding male and forming schools, members of the school would be related by at least $r = 0.25$.

3. Results

Although the mean number of alleles observed in the polymorphic loci was fairly high (3), the amount

Table 1. The enzymes and loci studied, Enzyme Commission (E.C.) numbers and buffers used in each locus.

Enzyme	Locus	E.C.	Buffer
Lactate dehydrogenase	*LDH**	1.1.1.27	II
Isocitrate dehydrogenase	*IDH**	1.1.1.42	I
Phosphoglucomutase	*PGM-1**	2.7.5.1	II
	*PGM-2**		
Esterase[1)]	*EST**	3.1.1.1	II
Peptidase[2)]	*PEP**	3.4.*.*	I
Aconitase	*ACO**	4.2.1.3	II
Phosphoglucose isomerase	*PGI**	5.3.1.9	I

[1)] 4-methylumbelliferryl acetate as substrate, [2)] glycyl-L-leucine as substrate.
Buffers: (I) Gel: 42.9 mmol l^{-1} TRIS, 4.2 mmol l^{-1} citrate, pH 8.4;
Tray: 135 mmol l^{-1} TRIS, 42.9 mmol l^{-1} citrate, pH 7.1 (Varvio-Aho & Pamilo 1980).
(II) Gel: 9 mmol l^{-1} TRIS, 3 mmol l^{-1} citrate, 1.2 mmol l^{-1} EDTA, pH 7;
Tray: 135 mmol l^{-1} TRIS, 44.5 mmol l^{-1} citrate, 1.2 mmol l^{-1} EDTA, pH 7 (Ayala *et al.* 1974).

of variation was generally low (Table 2), but well within the range observed in other studies on the three-spined stickleback (Buth & Haglund 1994). Only three loci had a frequency of the most common allele lower than 0.95 (Table 2). In the estimation of inbreeding and relatedness, however, the loci are weighted according to their information content, and the more inaccurate single locus estimates of the less polymorphic loci get less weight when the population averages are calculated (Queller & Goodnight 1989).

With one exception (*IDH**), the 95% confidence intervals of single locus estimates of relatedness included the zero value (Fig. 1) indicating no or very low relatedness within the schools. The confidence intervals of the three most informative loci (*PEP**, *EST**, *PGM-2**) were, however, quite wide. Especially with *est*, the accuracy of the estimate is not very high, the confidence interval including both zero and the expected value of $r = 0.25$ (Fig. 1). Nevertheless, with both *PEP** and *PGM-2** the upper 95% limit was clearly lower than the expected value of 0.25. In *IDH** the entire confidence interval was below the zero value, which presumably is due to the low polymorphism of the locus and thus its susceptibility to chance effects. Finally, the upper confidence limits of the population estimates (when S.E.'s jackknifed over both loci and groups) were clearly lower than 0.25. However, the intervals did not include the zero value, either (Fig. 1).

All single locus estimates of inbreeding were close to zero (in the three cases where the estimate differed from zero, it was significantly lower than zero; Table 3). Accordingly, the population average equalled zero indicating random mating in our study population (Table 3).

Table 2. Allele frequencies at eight allozyme loci in the study population of the three-spined stickleback. The frequencies were calculated weighting schools equally.

	Allele			
Locus	*a*	*b*	*c*	*d*
*LDH**	0.995	0.004	0.001	–
*IDH**	0.991	0.007	0.002	–
*PGM-1**	0.981	0.009	0.009	0.002
*PGM-2**	0.571	0.429	–	–
*EST**	0.934	0.060	0.001	0.005
*PEP**	0.893	0.107	–	–
*ACO**	0.990	0.006	0.004	–
*PGI**	0.964	0.035	0.001	–

4. Discussion

We hypothesised that schools of juvenile three-spined sticklebacks might consist of close relatives originating from the same nest. When the expected number of parents per nest is four or less (see Rico *et al.* 1991, 1992), it is reasonable to assume that individuals from the same nest are related to each

Table 3. Single locus and population estimates of inbreeding ($F \pm$ S.E.). *N* refers to the number of individuals sampled for each locus. The S.E.'s of the population estimates are shown when jackknifed both over loci (first row) and groups (second row). Test statistics for the deviation of the inbreeding estimates from the expected value ($F = 0$) are from *t*-tests.

Locus	*N*	*F*	df	*t*	*p*
*LDH**	397	– 0.003 ± 0.002	23	1.5	n.s.
*IDH**	397	– 0.007 ± 0.002	23	3.5	< 0.001
*PGM-1**	396	– 0.012 ± 0.005	23	2.4	< 0.05
*PGM-2**	334	0.103 ± 0.068	23	1.5	n.s.
*EST**	394	0.131 ± 0.095	23	1.4	n.s.
*PEP**	343	– 0.101 ± 0.033	23	3.1	< 0.01
*ACO**	390	– 0.006 ± 0.006	23	1.0	n.s.
*PGI**	396	0.013 ± 0.049	23	0.3	n.s.
Population	397	0.055 ± 0.048	7	1.1	n.s.
		0.055 ± 0.045	23	1.2	n.s.

other about as much as half sibs are. If sticklebacks associated with their nest mates when forming schools, similar levels of relatedness should also be found in the schools. Furthermore, if individuals discriminated between sibs and less related individuals (FitzGerald & Morrissette 1992), relatedness in a school could even be higher.

Our results show that members of the schools are less related than expected based on the assumptions above. In fact, the single locus estimates suggested very low or no relatedness at all among school mates. Furthermore, our expected relatedness, $r = 0.25$, was a conservative minimum estimate for relatedness within schools, because the above value was based on no reproductive skew among reproducing females and males. Skew is high in both sexes in the three-spined stickleback (Kedney *et al.* 1987, Rico *et al.* 1991, 1992, Goldschmidt *et al.* 1992), and in practise the expected relatedness would probably be closer to the full sibling value $r = 0.5$. So, despite the apparent opportunity of the young sticklebacks to stay with their close kin when forming schools, our results suggest that on average schools of stickleback juveniles are random samples from the genetic pool of the study population.

Since we only collected discrete schools, the lack of close relatedness within schools of young sticklebacks can not be due to collecting individuals from more than one school at time (e.g., Naish *et al.* 1993). There are alternative explanations, however. For instance predation might prevent longer existence of schools consisting of close kin. Our study area in the Baltic littoral is rich of piscivorous predators, both birds and fish. An attack by a predator may lead to a momentary scattering of the group members (Pitcher & Parrish 1993) and thus cause some individuals to separate from their original school and later on join another one. Therefore, even if individuals showed preference to associate with their kin in the first place, the above mechanism could readily lead to mixed schools of stickleback juveniles in the long run, especially if individuals have low probability to re-encounter after having been separated from each other. Young sticklebacks in the study population of Van Havre and FitzGerald (1988) and FitzGerald and Morrissette (1992) spend their first months in their natal tide pool. In such a habitat, individuals presumably have more opportunities to re-encounter kin than in the more wide and open sea littoral of our study area. The predation level in the pools may also be lower owing to the absence of other fish predators than sticklebacks from them (FitzGerald & Morrissette 1992).

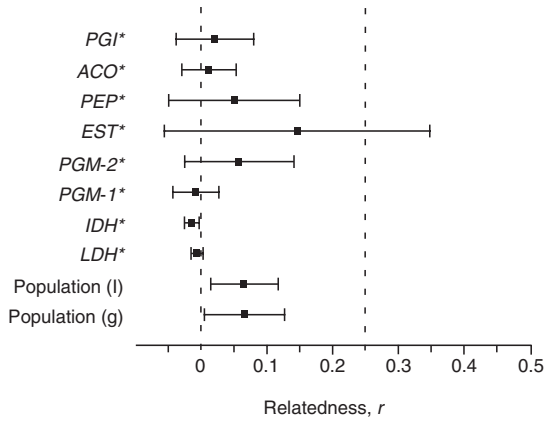

Fig. 1. Single locus and population estimates of relatedness (r) with their 95% confidence intervals. The confidence intervals of population estimates are shown with S.E.'s jackknifed both over loci (l) and groups (g). The dashed vertical lines indicate no relatedness, $r = 0$, and expected relatedness, $r = 0.25$, respectively.

Close relatives might also avoid forming aggregations with each other under certain ecological conditions (Giraldeau & Caraco 1993). For example, food limitation may result in severe within-group competition (Pulliam & Caraco 1984) and therefore grouping with non-relatives might be favoured. Furthermore, the lack of relatedness in stickleback schools could also follow from individuals not discriminating between kin and non-kin, schooling behaviour better reflecting the direct fitness benefits (Pitcher & Parrish 1993) rather than the kin-selected inclusive fitness benefits (Hamilton 1964). This is supported by the fact that juvenile stickleback schools in our study population are size-assorted (Peuhkuri *et al.* 1997), but not kin-structured. However, young sticklebacks were shown to prefer the company of their kin in laboratory studies by Van Havre and FitzGerald (1988) and FitzGerald and Morrissette (1992), suggesting that kin recognition occurs. At present, no data are available on kin recognition of sticklebacks from our study population, or any other population than the one studied by Van Havre and FitzGerald (1988) and

FitzGerald and Morrissette (1992). Therefore, kin recognition in the three-spined stickleback deserves further investigation in differing ecological contexts.

Acknowledgements: We thank S.-K. Juvonen and A. Laurila for help with the capture of sticklebacks and Tvärminne Zoological Station for providing good working facilities. A. Laurila, P. Pamilo, E. Ranta, L. Sundström and an anonymous referee provided valuable comments on the manuscript, and H. Rita gave us statistical advice. The study was funded by the Academy of Finland, Emil Aaltonen Foundation, University of Helsinki and NorFA.

References

Alexander, R. D. 1974: The evolution of social behavior. — Ann. Rev. Ecol. Syst. 5: 325–383.

Avise, J. C. & Shapiro, D. Y. 1986: Evaluating kinship of newly settled juveniles within social groups of the coral reef fish Anthias squamipinnis. — Evolution 40: 1051–1059.

Ayala, F. J., Valentine, J. W., Barr, L. G. & Zumwalt, G. S. 1974: Genetic variability in a temperate intertidal phoronid, Phoronopsis viridis. — Biochem. Gen. 11: 413–427.

Bertram, B. C. R. 1978: Living in groups: predators and prey. — In: Krebs, J. R. & Davies, N. B. (eds.), Behavioural ecology. An evolutionary approach: 64–96. Blackwell Sci. Publ., Oxford.

Brown, G. E. & Brown, J. A. 1993. Social dynamics in salmonid fishes: do kin make better neighbours? — Anim. Behav. 45: 863–871.

Brown, G. E., Brown, J. A. & Crosbie, A. M. 1993: Phenotypic matching in juvenile rainbow trout. — Anim. Behav. 46: 1223–1225.

Buth, D. G. & Haglund, T. R. 1994: Allozyme variation in the Gasterosteus aculeatus complex. — In: Bell, M. A. & Foster, S. A. (eds.), The evolutionary biology of the threespine stickleback: 61–84. Oxford Univ. Press, Oxford.

Clark, C. W. & Mangel, M. 1984: Foraging and flocking strategies: information in an uncertain environment. — Am. Nat. 123: 626–642.

Costa, J. T. III & Ross, K. G. 1993: Seasonal decline in intracolony genetic relatedness in eastern tent caterpillars: implications for social evolution. — Behav. Ecol. Sociobiol. 32: 47–54.

Crozier, R. H. & Pamilo, P. 1996: Evolution of Social Insect Colonies. — Oxford Univ. Press, Oxford.

Dowling, T. E. & Moore, W. S. 1986: Absence of population subdivision in the common shiner, Notropis cornutus (Cyprinidae). — Env. Biol. Fish. 15: 151–155.

Efron, B. & Tibshirani, R. J. 1993: An introduction to the bootstrap. — Monographs on statistics and applied probability, 57. Chapman & Hall, New York.

Ferguson, M. M. & Noakes, D. L. G. 1981: Social grouping and genetic variation in the common shiner Notropis cornutus. — Env. Biol. Fish. 6: 357–360.

FitzGerald, G. J. & Morrissette, J. 1992: Kin recognition and choice of shoal mates by threespine stickleback. — Ethol. Ecol. Evol. 4: 273–283.

Giraldeau, L.-A. & Caraco, T. 1993: Genetic relatedness and group size in an aggregation economy. — Evol. Ecol. 7: 429–438.

Goldschmidt, T., Foster, S. A. Sevenster, P. 1992: Internest distance and sneaking in the three-spined stickleback. — Anim. Behav. 44: 793–795.

Goodnight, K. F. & Queller, D. C. 1994: Relatedness 4.2. — Goodnight Software, Houston.

Hamilton, W. D. 1964: The genetical evolution of social behaviour I and II. — J. theor. Biol. 7: 1–52.

Harris, H. & Hopkinson, D. A. 1976: Handbook of enzyme electrophoresis in human genetics. — North Holland, Amsterdam.

Kedney, G. I., Boulé, V. & FitzGerald, G. J. 1987: The reproductive ecology of threespine sticklebacks breeding in fresh and brackish water. — In: Dadswell, M. J., Klauda, R. J., Moffitt, C. M., Saunders, R. L., Rulifson, R. A. & Cooper, J. E. (eds.), Common strategies of anadromous and catadromous fishes, American Fisheries Society Symposium No. 1: 151–161. Am. Fish. Soc., Maryland, Bethesda.

Magurran, A. E. 1990: The adaptive significance of schooling as an anti-predator defence in fish. — Ann. Zool. Fennici 27: 51–66.

Milinski, M., Lüthi, J. H., Eggler, R. & Parker, G. A. 1997: Cooperation under predation risk: experiments on costs and benefits. — Proc. R. Soc. Lond. B. 264: 831–837.

Naish, K.-A., Carvalho, G. R. & Pitcher, T. J. 1993: The genetic structure and microdistribution of shoals of Phoxinus phoxinus, the European minnow. — J. Fish Biol. 43(Suppl A): 75–89.

Olsén, K. H. 1989: Sibling recognition in juvenile Arctic charr (Salvelinus alpinus (L.)). — J. Fish Biol. 34: 571–581.

Olsén, K. H., Järvi, T. & Löf, A.-K. 1996: Aggressiveness and kinship in brown trout (Salmo trutta) parr. — Behav. Ecol. 7: 445–450.

Pamilo, P. 1989: Estimating relatedness in social groups. — Trends Ecol. Evol. 4: 353–355.

Pamilo, P. & Crozier, R. H. 1982: Measuring genetic relatedness in natural populations: methodology. — Theor. Pop. Biol. 21: 171–193.

Peuhkuri, N., Ranta, E. & Seppä, P. 1997: Size-assortative schooling in free-ranging sticklebacks. — Ethology 103: 318–324.

Pitcher, T. J. & Parrish, J. K. 1993: Functions of shoaling behaviour in teleosts. — In: Pitcher, T. J. (ed.), Behaviour of teleost fishes. 2 ed.: 363–439. Chapman & Hall, London.

Pulliam, H. R. & Caraco, T. 1984: Living in groups: is there an optimal group size? — In Krebs, J. R. & Davies, N. B. (eds.), Behavioural ecology. An evolutionary ap-

proach. 2 ed.: 122–147. Blackwell Sci. Publ., Oxford.
Pusey, A. E. & Packer, C. 1997. The ecology of relationships. — In: Krebs, J. R. & Davies, N. B. (eds.), Behavioural ecology. An evolutionary approach. 4 ed.: 254–283. Blackwell Sci. Publ., Oxford.
Queller, D. C. 1993: Genetic relatedness and its components in polygynous colonies of social wasps. — In: Keller, L. (ed.), Queen number and sociality in insects: 132–152. Oxford Univ. Press, Oxford.
Queller, D. C. & Goodnight, K. F. 1989: Estimating relatedness using genetic markers. — Evolution 43: 258–275.
Quinn, T. P. & Busack, C. A. 1985: Chemosensory recognition of siblings in juvenile coho salmon (Oncorhynchus kisutch). — Anim. Behav. 33: 51–56.
Quinn, T. P. & Hara, T. J. 1986: Sibling recognition and olfactory sensitivity in juvenile coho salmon (Oncorhynchus kisutch). — Can. J. Zool. 64: 921–925.
Ranta, E., Peuhkuri, N. & Laurila, A. 1994: A theoretical exploration of antipredatory and foraging factors promoting phenotype-assorted fish schools. — Ecoscience 1: 99–106.
Rico, C., Kuhnlein, U. & FitzGerald, G. J. 1991: Spawning patterns in the three-spined stickleback (Gasterosteus aculeatus L.): an evaluation by DNA fingerprinting. — J. Fish Biol. 39(Suppl. A): 151–158.
Rico, C., Kuhnlein, U. & FitzGerald, G. J. 1992: Male reproductive tactics in the threespine stickleback – an evaluation by DNA fingerprinting. — Mol. Ecol. 1: 79–87.
Van Havre, N. & FitzGerald, G. J. 1988: Shoaling and kin recognition in the threespine stickleback (Gasterosteus aculeatus L.). — Biol. Beh. 13: 190–201.
Varvio-Aho, S. & Pamilo, P. 1980: A new buffer system with wide applicability. — Isozyme Bull. 13: 114.
Whoriskey, F. G. & FitzGerald, G. J. 1993: Ecology of the threespine stickleback on the breeding grounds. — In: Bell, M. A. & Foster, S. A. (eds.), The evolutionary biology of the threespine stickleback: 188–206. Oxford Univ. Press, Oxford.
Wootton, R. J. 1991: Ecology of teleost fishes. — Chapman & Hall, London.
Wright, S. 1951: The genetical structure of populations. — Ann. Eugen. 15: 323–354.