Sea lamprey *Petromyzon marinus*: an exception to the rule of homing in anadromous fishes

John Waldman^{1,*}, Cheryl Grunwald²
and Isaac Wirgin²

¹Biology Department, Queens College, 65-30 Kissena Boulevard, Flushing, NY 11367, USA

²Department of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, USA

*Author for correspondence (john.waldman@qc.cuny.edu).

Anadromous fishes are believed to make regular circuits of migration in the sea before homing to their natal rivers. Sea lamprey *Petromyzon marinus* is an anadromous fish that is an exception to this life-history pattern. It also differs from other anadromous fishes in that its adult phase is parasitic, a feeding strategy that should make homing problematic for lamprey cohorts that become widely dispersed through transport by the diverse hosts they parasitize. We sequenced a portion of the mitochondrial DNA control region from sea lampreys collected from 11 North American east coast rivers to test for genetic evidence of homing. There were no significant differences ($\chi^2=235.1$, $p=0.401$) in haplotype frequencies among them, with almost 99 per cent of haplotypic diversity occurring within populations. These findings, together with concordant genetic results from other geographical regions and ancillary information on pheromonal communication, suggest that sea lamprey does not home but rather exhibits regional panmixia while using a novel 'suitable river' strategy to complete its life cycle.

Keywords: anadromous; homing; mitochondrial DNA; parasitism; pheromone

1. INTRODUCTION

Anadromous fishes undertake regular seasonal and life-stage-consistent migrations between freshwaters, where they begin life, and the sea. Anadromy occurs in less than 1 per cent of the world's fishes but it has evolved multiple times across a broad phylogenetic distribution (McDowall 2001). Some taxa in which anadromy are represented include petromyzontids, acipenserids, osmerids, salmonids, alosines and moronids. Anadromous fishes may have substantial osmotic, bioenergetic and predation-exposure costs in moving between the two environments, but they benefit from the generally reduced predation on early life stages in rivers and access to the greater trophic resources of marine waters (Gross 1987).

As a group, anadromous fishes are believed to show homing to their natal rivers (McDowall 2001). One

benefit of homing is that it allows individuals to return to habitats of established spawning success (McDowall 2001). Homing mechanisms have not been studied in all anadromous taxa, but in salmonids (Dittman & Quinn 1996), alosines (Dodson & Leggett 1974) and acipenserids (Boiko *et al.* 1993) it is completed with olfactory recognition of the natal river following imprinting prior to outmigration. Homing in anadromous fishes allows genetic differentiation among populations, even between neighbouring populations, through genetic drift and selection (DeWoody & Avise 2000). Thus, within-population variation is accompanied by significant among-population variation in anadromous fishes that home.

Evidence that sea lamprey *Petromyzon marinus* might not show homing fidelity emerged from Lake Huron of the Great Lakes watershed, where a tagging study of 555 outmigrating individuals from Devil River to Lake Huron recaptured 42 in-migrating spawning adults in four other rivers but none in Devil River (Bergstedt & Seelye 1995). To test whether this apparent absence of homing in its non-native landlocked range (Waldman *et al.* 2004) is a species-wide characteristic, we examined the population structure of anadromous sea lampreys in their native range collected from rivers within the Acadian and Virginian zoogeographic provinces, between Quebec and New York.

2. MATERIAL AND METHODS

Adult lampreys ($n=314$) were collected in rivers extending from St Lawrence River, Quebec, to Delaware River, New York (figure 1). Total DNA was isolated from fin clips stored in 95 per cent EtOH using proteinase K digestions in CTAB buffer (Waldman *et al.* 2006). A 511 bp portion of the mtDNA control was PCR amplified and 333 bp were sequenced in both directions using sea lamprey-specific primers (Waldman *et al.* 2004). Composite haplotypes were generated and their frequencies among collection sites were compared for statistical significance using a Monte Carlo-based χ^2 approach (Roff & Bentzen 1989). Haplotypic variation within versus among rivers was conducted using analysis of molecular variance (AMOVA; Excoffier *et al.* 1992). Sequence differences among haplotypes and GenBank accession numbers are available in the electronic supplementary materials.

3. RESULTS

There were no significant differences ($\chi^2=235.1$, $p=0.401$) in haplotype frequencies among these collections of sea lampreys as seen in other anadromous fishes across all or part of this range that home, including Atlantic salmon *Salmo salar* (King *et al.* 2001), striped bass *Morone saxatilis* (Wirgin *et al.* 1997), Atlantic sturgeon *Acipenser oxyrinchus* (Grunwald *et al.* 2007) and American shad *Alosa sapidissima* (Bentzen *et al.* 1989). AMOVA showed extremely low haplotypic variation among sea lamprey collection sites (1.3%); virtually all haplotypic diversity, almost 99 per cent, occurred within populations.

4. DISCUSSION

Our results indicate that sea lampreys from western Atlantic rivers, even across two zoogeographic provinces, exhibit regional panmixia. This corresponds with our findings of no significant differences ($p>0.05$) in haplotypic frequencies among collections in other discrete regions, including native landlocked populations in Lake Ontario (three rivers; Waldman *et al.* 2004), in Lake Champlain (four rivers;

Figure 1. Mitochondrial DNA haplotype proportions in sea lamprey collections from 11 North American east coast rivers. Sample sizes in parentheses.

Waldman *et al.* 2006) and between two rivers in Portugal ($p=0.8643$; Rodríguez-Muñoz *et al.* 2004). Moreover, it is congruent with findings from nuclear DNA microsatellite analyses for Atlantic coast anadromous sea lamprey populations and among populations in Lakes Ontario and Champlain (Bryan *et al.* 2005). We hypothesize that this panmixia is one outcome of an alternative life-history strategy from that employed by other anadromous fishes, and that this strategy is directly allied with its parasitism.

Other anadromous fishes such as salmon (Groot & Margolis 1991), striped bass (Boreman & Lewis 1987) and shads (Dadswell *et al.* 1987) maintain regular circuits of migration in the sea, which facilitate feeding in productive marine waters together with coordinated returns to their natal rivers for reproduction. Sea lampreys, however, leave their natal rivers and feed sanguivorously for as much as 3 or 4 years, for several days to over two weeks per host, during which time they are transported by their hosts' movements (Beamish 1980). The spectrum of potential hosts in marine waters is extensive; documented prey off North America include but are not limited to pelagic fishes such as Atlantic salmon, bluefish *Pomatomus saltatrix*, Atlantic mackerel *Scomber scombrus*, menhaden *Brevoortia tyrannus*, bluefin tuna *Thunnus thynnus*, swordfish *Xiphias gladius* and basking shark *Cetorhinus maximus*; benthic fishes such as Atlantic cod *Gadus morhua*, haddock *Melanogrammus aeglefinus*, hakes *Urophycis* spp., Atlantic sturgeon; and cetaceans (Collette & Klein-MacPhee 2002). There is little commonality among these and other possible hosts in seasonal and annual movement patterns, many of which are considerable, and include much of the western Atlantic Ocean when summed across species.

Probably owing to this non-specificity in host selection and its consequent implications for transport, sea lamprey has been found in the ocean from the surface to over 4000 m in depth (Beamish 1980). The horizontal spatial consequences of such diverse feeding opportunities are even more profound. Unlike for an outmigrating cohort of a non-parasitic anadromous fish, which may maintain cohesion by following a migratory circuit, a cohort of sea lamprey that emigrates from a river, after completing its marine feeding phase on a diverse suite of hosts, should be widely dispersed in ocean waters.

Such a broad geographical distribution, in the absence of homing, raises the question of how sea lampreys locate suitable spawning rivers inasmuch as many drainages do not provide both appropriate spawning and rearing habitats (Beamish 1980). Sea lampreys build nests only in shallow riffle areas with rubble of a particular size range, but their larvae inhabit sandy and silty backwaters where they develop in burrows for 2–8 years (Quintella *et al.* 2003) before transforming to the adult phase and outmigrating. The subsequent choice of river for spawning is critical in that sea lampreys expend considerable energy reserves before and during spawning (Beamish 1979) and then die shortly thereafter. However, many rivers are unsuitable for sea lamprey reproduction. In the Great Lakes, where sea lampreys became very abundant, only 8 per cent of tributaries have supported populations of sea lamprey larvae (Morman *et al.* 1980).

Strong evidence exists that chemical means afford an alternative 'suitable river' strategy to homing based on recognition of the presence of conspecifics ('kin recognition'; McDowall 2001). Larvae have been

shown to release a bile acid-based pheromone detectable to adults in marine waters (Bjerselius et al. 2000), with individual larvae activating approximately 400 l water h⁻¹ (Sorensen et al. 2003). Adult males that ascend to the gravel-bed spawning grounds then use powerful pheromones to communicate their presence to females downstream (Li et al. 2002).

Our genetic results, considered with ancillary information, suggest that we have identified a new relationship among two fundamental life-history characteristics, i.e. the presence of parasitism has coevolved with an alternative reproductive strategy for an anadromous fish species, and the use of a suitable river strategy, instead of homing. Both strategies involve olfactory certification of the reliability of the spawning habitat prior to bioenergetically costly upriver migration, but the one used by salmonids and alosines is based on within-individual 'memory', whereas the other is based on contemporaneous chemical signalling.

Our data combined with the information on chemical communication also suggest a strategy for sea lamprey control. Destructive parasitism by sea lampreys within their non-native range in the Great Lakes and also in Lakes Ontario and Champlain has caused substantial economic damage to fisheries (Holeck et al. 2004). In the absence of homing, the application of sea lamprey pheromones to rivers believed to be unsuitable for their spawning and nursery purposes might divert reproductive effort unproductively with little risk of establishing a new population.

Approximately half a dozen marine lamprey species exist elsewhere in the world (Nelson 1976). We predict that this same relationship holds for them too, as indicated for Pacific lamprey *Entosphenus tridentatus* where no significant haplotypic differences were seen among collections from 81 rivers across six zoogeographic regions between British Columbia and California, and where within-river differences also accounted for 99 per cent of the variation (Goodman et al. 2008). The life-history pattern identified for marine lampreys appears unique for diadromous fishes, but we note that it is the converse of catadromous anguillid eels that too are panmictic, but where for eels disaggregation occurs as individuals enter freshwaters, not as they exit them.

This work was partially supported by a National Oceanic and Atmospheric Administration (NOAA) award to the Research Foundation of the State University of New York for New York Sea Grant. The US Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. Additional resources were provided by NIEHS Center Grant ES00260.

- Beamish, F. W. H. 1979 Migration and spawning energetics of the anadromous sea lamprey, *Petromyzon marinus*. *Environ. Biol. Fish.* **4**, 3–7. (doi:10.1007/BF00005922)
- Beamish, F. W. H. 1980 Biology of the North American anadromous sea lamprey, *Petromyzon marinus*. *Can. J. Fish. Aquat. Sci.* **37**, 1924–1943. (doi:10.1139/f80-233)
- Bentzen, P., Brown, G. C. & Leggett, W. C. 1989 Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). *Can. J. Fish. Aquat. Sci.* **7**, 1446–1454.
- Bergstedt, R. A. & Seelye, J. G. 1995 Evidence for a lack of homing by sea lampreys. *Trans. Am. Fish. Soc.* **124**, 235–239. (doi:10.1577/1548-8659(1995)124<0235:EFLOHB>2.3.CO;2)
- Bjerselius, R., Li, W., Teeter, J. H., Seelye, J. G., Johnsen, P. B., Maniak, P. J., Grant, G. C., Polkinghorne, C. N. & Sorensen, P. W. 2000 Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone. *Can. J. Fish. Aquat. Sci.* **57**, 557–569. (doi:10.1139/cjfas-57-3-557)
- Boiko, N. E., Grigoryan, R. A. & Chichachev, A. S. 1993 Olfactory imprinting in juveniles of Russian sturgeon, *Acipenser guldenstadti*. *J. Evol. Biochem. Physiol.* **29**, 509–514.
- Boreman, J. & Lewis, R. R. 1987 Atlantic coastal migration of striped bass. *Am. Fish. Soc. Symp.* **1**, 331–339.
- Bryan, M. B., Zalinski, D., Filcek, K. B., Libants, S., Li, W. & Scribner, K. T. 2005 Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. *Mol. Ecol.* **14**, 3757–3773. (doi:10.1111/j.1365-294X.2005.02716.x)
- Collette, B. B. & Klein-MacPhee, G. 2002 *Bigelow and Schroeder's fishes of the Gulf of Maine*. Washington, DC: Smithsonian Institution Press.
- Dadswell, M. J., Melvin, G. D., Williams, P. J. & Themelis, D. E. 1987 Influences of origin, life history, and chance on the Atlantic coast migration of American shad. *Am. Fish. Soc. Symp.* **1**, 313–330.
- DeWoody, J. A. & Avise, J. C. 2000 Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *J. Fish Biol.* **56**, 461–473. (doi:10.1111/j.1095-8649.2000.tb00748.x)
- Dittman, A. & Quinn, T. 1996 Homing in Pacific salmon: mechanisms and ecological basis. *J. Exp. Biol.* **199**, 83–91.
- Dodson, J. J. & Leggett, W. C. 1974 Role of olfaction and vision in the behavior of American shad (*Alosa sapidissima*) homing to the Connecticut River from Long Island Sound. *J. Fish. Res. Board Can.* **31**, 1607–1619.
- Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.
- Goodman, D., Reid, S., Docker, M. F., Haas, G. R. & Kinziger, A. P. 2008 Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). *J. Fish Biol.* **72**, 400–417. (doi:10.1111/j.1095-8649.2007.01731.x)
- Groot, C. & Margolis, L. (eds) 1991 *Pacific salmon life histories*. Vancouver, Canada: University of British Columbia Press.
- Gross, M. 1987 The evolution of diadromy in fishes. *Am. Fish. Soc. Symp.* **1**, 14–25.
- Grunwald, C., Maceda, L., Waldman, J., Stabile, J. & Wirgin, I. 2007 Conservation of Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus*: delineation of stock structure and distinct population segments. *Conserv. Genet.* **8**, 1566–1572.
- Holeck, K. T., Mills, E. L., MacIsaac, H. J., Dochoda, M. R., Colautti, R. I. & Ricciardi, A. 2004 Bridging troubled

- waters: biological invasions, transoceanic shipping, and the Laurentian Great Lakes. *Bioscience* **54**, 919–929. (doi:10.1641/0006-3568(2004)054[0919:BTWBIT]2.0.CO;2)
- King, T. L., Kalinowski, S. T., Schill, W. B., Spidle, A. P. & Lubinski, B. A. 2001 Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. *Mol. Ecol.* **10**, 807–821. (doi:10.1046/j.1365-294X.2001.01231.x)
- Li, W., Scott, A. P., Siefkes, M. J., Yan, H., Liu, Q., Yun, S.-S. & Gage, D. A. 2002 Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* **296**, 138–141. (doi:10.1126/science.1067797)
- McDowall, R. M. 2001 Anadromy and homing: two life-history traits with adaptive synergies in salmonid fishes? *Fish Fisheries* **2**, 78–85. (doi:10.1046/j.1467-2979.2001.00036.x)
- Morman, R. H., Cuddy, D. W. & Rugen, P. C. 1980 Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. *Can. J. Fish. Aquat. Sci.* **37**, 1811–1826. (doi:10.1139/f80-224)
- Nelson, J. S. 1976 *Fishes of the world*. New York, NY: Wiley.
- Quintella, B. R., Andrade, N. O. & Almeida, P. R. 2003 Distribution, larval stage duration and growth of the sea lamprey ammocoetes, *Petromyzon marinus* L., in a highly modified river basin. *Ecol. Freshw. Fish* **12**, 286–293. (doi:10.1046/j.1600-0633.2002.00030.x)
- Rodríguez-Muñoz, R., Waldman, J. R., Grunwald, C., Roy, N. K. & Wirgin, I. 2004 Mitochondrial DNA variation in sea lamprey between North American and Spanish rivers. *J. Fish Biol.* **64**, 783–787. (doi:10.1111/j.1095-8649.2004.00334.x)
- Roff, D. A. & Bentzen, P. 1989 The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol. Biol. Evol.* **6**, 539–545.
- Sorensen, P. W., Vrieze, L. A. & Fine, J. M. 2003 A multi-component migratory pheromone in the sea lamprey. *Fish Phys. Biochem.* **28**, 253–257. (doi:10.1023/B:FISH.0000030545.39156.2b)
- Waldman, J. R., Grunwald, C., Roy, N. K. & Wirgin, I. I. 2004 Mitochondrial DNA analysis indicates sea lamprey (*Petromyzon marinus*) indigenous to Lake Ontario. *Trans. Am. Fish. Soc.* **133**, 950–960. (doi:10.1577/T03-104.1)
- Waldman, J. R., Grunwald, C. & Wirgin, I. 2006 Evaluation of the native status of sea lamprey *Petromyzon marinus* in Lake Champlain based on mitochondrial DNA sequencing analysis. *Trans. Am. Fish. Soc.* **135**, 1076–1085. (doi:10.1577/T05-055.1)
- Wirgin, I. I., Waldman, J. R., Maceda, L., Stabile, J. & Vecchio, V. J. 1997 Mixed-stock analysis of Atlantic coast striped bass using nuclear DNA and mitochondrial DNA markers. *Can. J. Fish. Aquat. Sci.* **54**, 2814–2826. (doi:10.1139/cjfas-54-12-2814)