# The origin of introduced rainbow trout (*Oncorhynchus mykiss*) in the Santa Cruz River, Patagonia, Argentina, as inferred from mitochondrial DNA

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**Abstract:** Rainbow trout (*Oncorhynchus mykiss*) was first introduced into Argentinean Patagonia, the southernmost region of South America, from the United States in 1904 and at present constitutes the most conspicuous freshwater fish in lakes and rivers of the region. The Santa Cruz River in Southern Patagonia is the only river in the world where a self-sustained population of introduced rainbow trout is known to have developed an anadromous run. In this study, we examined mtDNA sequence variation to identify the source of Santa Cruz River rainbow trout, providing a historical framework to interpret the processes underlying phenotypic variation and structure of Patagonian populations. The Santa Cruz River may harbor distinct North American stocks of rainbow trout, widely distributed around the world during the late 19th and early 20th centuries, but today threatened after decades of habitat loss, species introduction, and introgression from alien stocks. The mtDNA sequence data revealed that the most likely origin for wild anadromous and nonanadromous fish was the McCloud River in California. Meanwhile, a local hatchery stock, representative of rainbow trout introduced from Denmark after 1950 and widely stocked ever since throughout Patagonia, most probably originated from multiple lineages from western North America, including non-Californian populations.

**Résumé :** La truite arc-en-ciel (*Oncorhynchus mykiss*) a été introduite en Patagonie argentine, la région la plus australe de l'Amérique du Sud, depuis les États-Unis en 1904 et elle est actuellement le poisson d'eau douce le plus en évidence dans les lacs et rivières de la région. La rivière Santa Crux en Patagonie du Sud est la seule rivière au monde dans laquelle une population autosuffisante de truites arc-en-ciel introduites ait développé une migration anadrome. Notre étude examine la variation des séquences de l'ADN mitochondrial (ADNmt) afin d'identifier l'origine des truites arc-en-ciel de la rivière Santa Crux et elle procure ainsi un cadre historique pour l'interprétation des processus sous-jacents à la variation phénotypique et à la structure des populations de Patagonie. La rivière Santa Crux peut contenir des stocks différents de truites arc-en-ciel nord-américains qui ont été largement répandus à travers le globe à la fin du 19$^{e}$ siècle et au début du 20$^{e}$ siècle, mais qui sont aujourd'hui menacés après des décennies de pertes d'habitats, d'introductions d'espèces et d'introgressions avec les stocks étrangers. D'après les données de séquences d'ADNmt, l'origine la plus probable des poissons sauvages anadromes et non anadromes est la rivière McCloud de Californie. De plus, un stock local de pisciculture, typique des truites arc-en-ciel introduites du Danemark après 1950 et ensemencées depuis lors partout en Patagonie, provient très probablement de multiples lignées de l'ouest de l'Amérique du Nord, y compris de populations d'ailleurs que la Californie.

[Traduit par la Rédaction]

## Introduction

Salmon and trout have been transplanted to habitats throughout the world and self-sustaining populations have been successfully established globally, with the exception of Antarctica (MacCrimmon 1971; Quinn et al. 1996; Nielsen 1996). Rainbow trout (*Oncorhynchus mykiss*) was first introduced into Argentinean Patagonia, the southernmost region of South America, at the turn of the twentieth century and eventually became the most conspicuous freshwater species in major river basins of the region (Pascual et al. 2002*b*).

Like all other known introduced rainbow trout around the world, typical Patagonian fish remain in fresh water throughout their entire life cycle, with a life history similar to that of resident populations in rivers and head lakes in western North America (Wydosky and Whitney 1979). The Santa Cruz River in Patagonia (50°S) is the only drainage in the world where introduced rainbow trout are known to have

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developed partially migratory populations composed of individuals exhibiting a marine migratory phase, so-called steelhead, and strictly freshwater fish that remain resident in their native stream (Pascual et al. 2001).

As in many other salmonid populations with this dual anadromous–nonanadromous life history, the way and extent to which the two ecotypes intermingle in the Santa Cruz is uncertain. Genetic analyses based on microsatellite loci revealed that the anadromous form is genetically indistinguishable from main-stem resident trout (Pascual et al. 2001), suggesting that significant gene flow occurs between the two forms.

Whether the introduced fish were in effect anadromous or anadromy arose in situ remains unknown (Behnke 2002; Pascual et al. 2002*a*). We also ignore the specific mechanisms underlying the expression of alternative life histories in the Santa Cruz, i.e., a genetic polymorphism, a genetically determined developmental threshold (i.e., the link between individual growth performance and anadromy or nonanadromy; Thorpe et al. 1998), or an entirely environmental effect. At this point, there are critical aspects regarding the environmental versus genetics bases of life history variation in Santa Cruz River rainbow trout that we do not know.

A logical first step to start elucidating the bases of life history variation in Patagonian rainbow trout, in particular, the development of anadromy, is to assess their genetic legacy through the identification of the parental sources. Poor historical bookkeeping and complex ancestry have made it difficult to address this issue from transplant records alone. The Santa Cruz River, as well as all other rivers throughout Patagonia, received rainbow trout from two main sources at different times. Between 1904 and 1910, rainbow trout ova were imported from the United States (US), most likely derived from rainbow trout and steelhead from locations in northern California or southern Oregon (Pascual et al. 2001, 2002*a*; Behnke 2002). After the 1930s, and particularly after the 1950s when fish transplants within the region became more common, all rainbow trout plantings were based on new stocks imported from Germany and Denmark (Baigún and Quirós 1985). However, the Santa Cruz River has had a history largely independent from that of more northerly Patagonia locations, with only occasional introductions after 1920 (Pascual et al. 2001, 2002*a*). Thus, presumably, wild populations in this river were mostly derived from the early shipments from the United States.

Mitochondrial DNA (mtDNA) has proven very successful for identifying the origins of several introduced salmonid populations and for assessing genetic differences between contemporary wild and introduced populations (Quinn et al. 1996; Burger et al. 2000). In this paper, we use mtDNA sequence variation to identify the founding populations of Santa Cruz River rainbow trout. We start by analyzing mtDNA sequences of both resident and migratory fish. We include in the analysis fish from a local hatchery, which was founded with European trouts widely stocked around the region after 1950. We then build and apply a probabilistic model of random survival and reproduction of individual fish to calculate the likelihood that wild Santa Cruz fish had originated from a collection of candidate North American stocks. Finally, we discuss the merits of the techniques applied to evaluate the relative contribution of pre-1950 transplants from US stocks and post-1950 transplants from Danish stocks to wild populations of rainbow trout throughout Patagonia.

## Methods

### Transplant history of rainbow trout in the Santa Cruz River

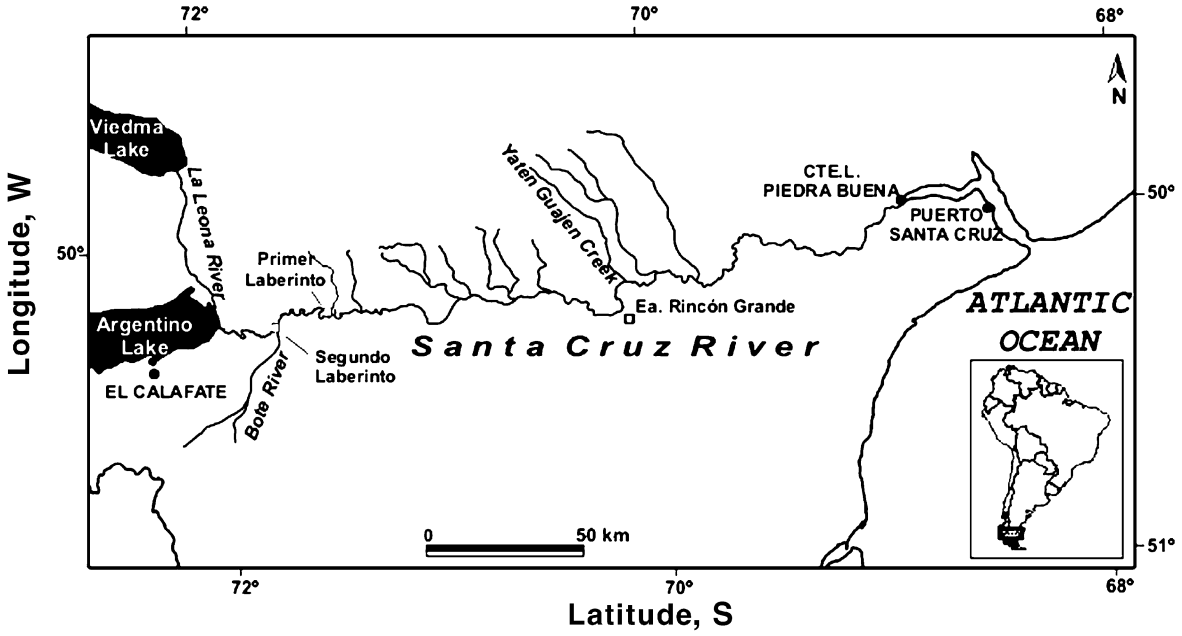
From 1904 to 1910, several consignments of rainbow trout embryos arrived at Argentina, mainly from the United States, with only occasional importations from European countries, such as France and Germany (Tulian 1908; Marini and Mastrarrigo 1963; Behnke 2002). Between 1906 and 1910, a total of 105 000 rainbow trout ova collected in the United States were shipped to the Santa Cruz River: 25 000 in 1906, 30 000 in 1908, and 50 000 in 1909. The 1908 shipment was completely lost, but the other two consignments were successfully hatched and planted in the river, with comparable losses all through (about 65%; Tulian 1908; Marini and Mastrarrigo 1963). For practical purposes, therefore, the number of eggs from the parental populations giving rise to the Santa Cruz stock was 75 000. The most likely origin of these eggs was the Baird Station on the McCloud River, California (Pascual et al. 2001). However, they may as well have come from steelhead and rainbow trout in alternative northern California and southern Oregon locations (Behnke 2002; Pascual et al. 2002*a*).

Rainbow trout introductions into Argentina intensified after the 1950, this time based on stocks from Denmark (Pillay 1969; MacCrimmon 1971) and maintained by the Bariloche Hatchery in Northern Patagonia. By that time, Bariloche became the main center of salmonid propagation in Argentinean waters, contributing to the distribution of these new stocks throughout the 1950s, 1960s, and 1970s. Danish stocks of rainbow trout have a complex ancestry; multiple lineages from California, Michigan, Canada, New Zealand, and France appear to have contributed to their foundation (MacCrimmon 1971). Small consignments of these fish (<2000 embryos) arrived at the Santa Cruz River from Bariloche in the 1970s and were planted in second- to third-order tributaries flowing into the upper basin (Fig. 1). Finally, in 1991, the Piedra Buena Hatchery was built on the lower Santa Cruz River (Fig. 1). The fish used to found this hatchery's broodstock also came from Danish fish, as those kept by the Bariloche Hatchery. Although fish of this hatchery are not used for stocking the river, escapes are likely, so that some introgression with wild fish might occur (Pascual et al. 2001). In any case, these fish provide a representative group of known Danish origin with which to contrast the genetic structure of Santa Cruz River wild fish.

### Study localities

The upper Santa Cruz basin is dominated by two large glacial-fed lakes, Viedma and Argentino, that form the Santa Cruz River. The main stem river has an average flow of 690 $m^3 \cdot s^{-1}$ and extends for 382 km across the Patagonian plateau draining into the Atlantic Ocean (Fig. 1). Landlocked populations of rainbow trout inhabit most of the second- to third-order tributaries that feed the head lakes; few springs and small tributaries enter the main-stem river, none of them significant from the point of view of their trout populations.

**Fig. 1.** Location of the Santa Cruz River and tributaries in Southern Patagonia, Argentina, including the localities discussed (Primer Laberinto, Segundo Laberinto, Ea. Rincón Grande, and Piedra Buena).




We restricted our analysis to the main-stem river populations, which, as revealed by a telemetry study, is the domain of the anadromous rainbow trout and of the resident fish to whom they are most likely related (Riva Rossi et al. 2003).

Adult anadromous and resident rainbow trout were caught by hook and line and by gill nets between 2000 and 2002 in April and September along the main-stem Santa Cruz River (Fig. 1). Sampling locations were based on spawning and fishing abundances documented in previous surveys and consisted of two river reaches located in the upper course ("Primer Laberinto" and "Segundo Laberinto"), one in the middle course ("Ea. Rincón Grande"), and one in the lower course ("Cte. L. Piedra Buena" City). At each locality, tissue samples were obtained from fish of each ecotype. From a total of 182 wild fish captured, 20 were successfully sequenced: five individuals of each ecotype from the upper course, three resident fish from the middle course, and three anadromous and five resident fish from the lower course. Direct inspection of external characteristics and scale pattern analysis were used to distinguish anadromous from nonanadromous fish (Pascual et al. 2001). Also, fin clips were obtained from five spawners from the Piedra Buena Hatchery broodstock.

## DNA techniques

Whole genomic DNA was extracted from alcohol-preserved fin tissue by means of a sodium chloride extraction of proteins followed by ispropylic alcohol precipitation of DNA (Miller et al. 1988). The polymerase chain reaction (PCR) was used to amplify a segment of the mitochondrial genome containing 188 base pairs (bp) of the *O. mykiss* control region and 5 bp of the adjacent phenylalanine tRNA gene using primers S-phe (5′-CTTTAGTTAAGCTACG-3′) and P2 (5′-TGTTAAACCCCTAAACCAG-3′) (Nielsen et al. 1994). Nomenclature for mtDNA control region haplotypes follow those given in Nielsen et al. (1997*a*, 1998). Amplifications were conducted in a total volume of 50 μL containing 1× reaction buffer, 2 mmol $MgCl_2 \cdot L^{-1}$, 80 μmol deoxynucleoside triphosphate$\cdot L^{-1}$ (20 μmol$\cdot L^{-1}$ each), 0.08 unit of Promega Taq DNA polymerase, and approximately 100 ng of DNA. PCR cycling conditions consisted of an initial denaturing step of 94 °C for 2 min followed by 30 cycles of 30 s at 94 °C, 30 s at 50 °C, and 30 s at 72 °C. A final elongation step of 2 min at 72 °C ended the cycle. After the initial PCR, amplified fragments were evaluated via electrophoresis of 5 μL of the PCR product from each reaction in a 3.5% nondenaturing acrylamide gel, silver stained following Sanguinetti et al. (1994). For specimens that exhibited successful amplifications, the remaining PCR product was then purified with Sephardex spin columns for cycle sequencing. The nucleotide sequences were determined with the dideoxy chain termination method using an ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin–Elmer Corp., Norwalk, Conn.). The samples were analyzed with an automated DNA sequencer (ABI PRISM 377). Control region sequences of Santa Cruz River *O. mykiss* revealed no length or sequence variation, making sequence alignment straightforward.

## Data analysis

Sequences were assembled based on overlapping regions using Sequences Navigator (Applied Biosystems Inc. version 1.0.1) and sequence alignment was performed using CLUSTAL W (Thompson et al. 1994). Santa Cruz fish mtDNA control region sequences were aligned against those previously published by Nielsen et al. (1994) from California (ST1-14) and compared among the ecotypes and localities surveyed in this study and with those rainbow trout sequences available in the literature (e.g., Nielsen et al. 1994; Bagley and Gall 1998; McCusker et al. 2000).

## Model of haplotype frequencies

All 20 Santa Cruz wild fish analyzed had the same haplo-

type (ST1, Nielsen et al. 1994; details in Results), suggesting either that they descended from a monomorphic population, that the population became fixed for haplotype ST1 during establishment and colonization, or that not all population haplotypes were represented in our sample. We thus developed an ad hoc model to evaluate the likelihood of ending with an all-ST1 sample given that the stock of origin was nonmonomorphic. We consider three processes that, starting with a nonmonomorphic maternal stock, could lead to an all-ST1 sample: the sampling of females from the donor population that produced the eggs imported (founder effect), the mortality between eggs and reproductive fish contributing to establish the new stock (postfounding drift), and the chance of missing population haplotypes during our sampling process (sampling effect).

Each of these three processes can be viewed as sampling from a finite population, which is most properly modeled by a hypergeometric distribution. For the sample sizes and probabilities used in our analysis, the binomial distribution approximates the hypergeometric well. We therefore opted for computational simplicity and modeled the foundation of Santa Cruz populations as a chain of three binomial processes.

The number of donor females, different females that could have contributed to the Santa Cruz River stock, *F*, is calculated as

$$(1) \qquad F = \frac{F}{\text{fec}}$$

where *E* is the number of eggs imported and "fec" are putative values for average female fecundity. Assuming that the maternal females were randomly drawn from a particular population, we modeled the number of ST1 eggs effectively extracted from it and imported into Argentina, $E^{\text{ST1}}$, as a binomial process:

$$(2) \qquad E^{\text{ST1}} \approx \text{fec} \cdot \text{Bin}(F, \phi)$$

where $\phi$ is the frequency of the ST1 haplotype in the original population. The postintroduction mortality from eggs to founding fish, *W*, i.e., fish that effectively contributed to the Santa Cruz stock, is simply modeled as

$$(3) \qquad W = \text{surv} \cdot E$$

where "surv" are putative values of survival rate from eggs to founding fish. The number of ST1 fish in this founding stock is

$$(4) \qquad W^{\text{ST1}} \approx \text{Bin}\left(W, \frac{E^{\text{ST1}}}{E}\right)$$

where $E^{\text{ST1}}/E$ is the proportion of ST1 eggs effectively imported as modeled in eq. 2. The number of ST1 fish in the sample taken from the present population ($S^{\text{ST1}}$) is

$$(5) \qquad S^{\text{ST1}} \approx \text{Bin}\left(n, \frac{W^{\text{ST1}}}{W}\right)$$

where *n* is the sample size and $W^{ST1}/W$ is the proportion of ST1 individuals among the founding fish. It is assumed that the frequency of ST1 currently observed in the population is well represented by that of the founding fish. In other words, we assumed that there was a single, primeval bottleneck associated with initial establishment, after which the population expanded rapidly enough for the frequency of ST1 to remain reasonably unchanged.

The probability of obtaining and all-ST1 sample from the present population is

$$(6) \qquad P(S^{\text{ST1}} = n \mid W, W^{\text{ST1}}) = \left(\frac{W^{\text{ST1}}}{W}\right)$$

Finally, for given founding stock ($\phi$ is the frequency of ST1 in the maternal population), average fecundity (i.e., or number of donor females (eq. 1)), egg to founding fish survival (i.e., or number of founding wild fish (eq. 3)), and sample size (*n*), the probability of obtaining an all-ST1 sample is given by integrating eq. 6 over all possible outcomes of eqs. 4 and 2:

$$(7) \qquad P(S^{\text{ST1}} = n \mid E, W) = \sum_{E^{\text{ST1}}} \left( P(E^{\text{ST1}}) \left( \sum_{W^{\text{ST1}}} P(W^{\text{ST1}}) P(S^{\text{ST1}} = n \mid E, E^{\text{ST1}}, W, W^{\text{ST1}}) \right) \right)$$

The number of eggs imported, *E*, was set to 75 000. We used an array of values for "fec" between 500 (low fecundity) and 4500 (high fecundity), considering 2800 to be an average fecundity for typical Sacramento River rainbow trout stocks (Carlander 1969). These values correspond to a range of 17–150 donor females. We used values of $\phi$ consistent with the frequency of haplotype ST1 in different candidate donor populations of Santa Cruz River fish (Table 1). We used values of "surv" between 0.00006 and 0.0029, corresponding to founding population sizes of 5 (very low survival) to 215 fish (high survival). Finally, we used a sample size *n* of 20, the number of wild fish sequenced in this study.

We did not consider in our model the chance of missing low-frequency population haplotypes during our sampling process. While this probability may not be unimportant for sample sizes of less than 10 individuals and frequencies of 0.85, it becomes low for sample sizes of 20 individuals. We therefore preferred to accept a small bias and avoid the need for the much more intensive calculations demanded by including three nested conditional probabilities in our model.

## Results

Sequence data revealed that all Santa Cruz River fish, both anadromous and resident, had the ST1 haplotype described by Nielsen et al. (1994). Hatchery fish, on the other hand, were genetically different from wild fish. Only one of the five fish examined had haplotype ST1, while the remaining four fish had haplotypes ST3 and ST9 in similar proportions. Each of these haplotypes differed by only a single transitional base change from haplotype ST1 (G → A) at positions 1109 (ST3) and 1147 (ST9). All of these mtDNA haplotypes were previously reported by Nielsen et al. (1994, 1997*b*, 1998) and Bagley and Gall (1998) in rainbow trout populations from California and by McCusker et al. (2000) in populations from British Columbia.

Mitochondrial DNA haplotype ST1 is dominant in steel-

**Table 1.** Haplotype distribution in rainbow trout populations throughout California.

| Stock | Life history ecotype | Haplotype | Haplotype frequency (%) |
|---|---|---|---|
| McCloud redband trout | Resident (anadromous ancestry) | ST1 | 100 |
| Northern California | | | |
| Eel and Sacramento rivers | Coastal steelhead | ST1 | 83 |
| | | ST3 | 9 |
| | | ST5 | 4 |
| | | ST8 | 4 |
| Central California coast | | | |
| Russian River | Steelhead | ST3 | 41 |
| | | ST1 | 40 |
| | | ST5 | 15 |
| | | ST8 | 4 |
| Upper Sacramento River | Steelhead | ST3 | 62 |
| | | ST1 | 21 |
| | | ST2 | 14 |
| | | ST5 | 3 |
| | Resident | STH3 | >60 |

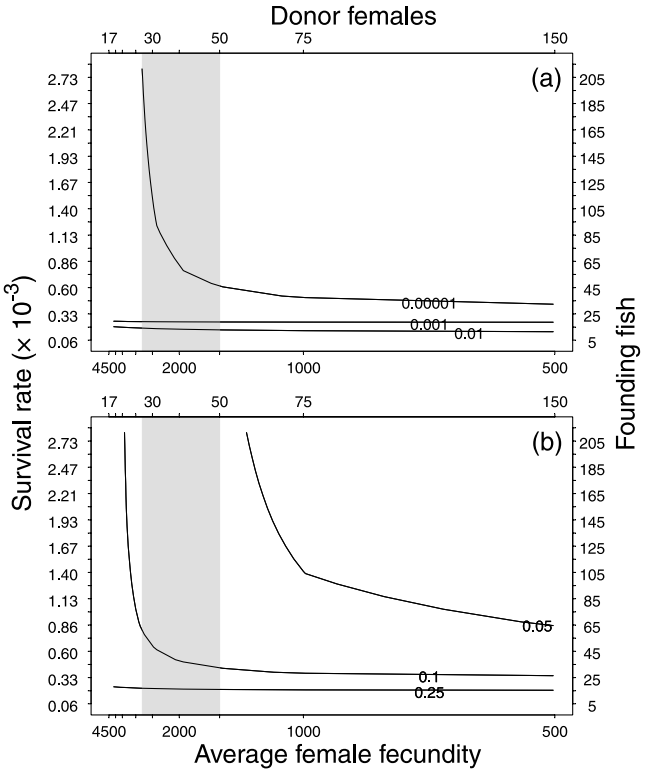
**Note:** Only those populations regarded as the putative parental sources for Santa Cruz River rainbow trout are included. Frequency data taken from Nielsen (1996) and Nielsen et al. (1994).

head populations from the Sacramento and Eel rivers in northern California but among the putative parental stocks was found to be monomorphic only in the McCloud River rainbow trout (Table 1) and in the Río Santo Domingo rainbow trout populations from Baja California (Nielsen et al. 1997*b*, 1998, 1999). We discard this last stock as a candidate source of Patagonian fish because Baja California trout did not contribute to fish culture at the time of the introductions.

Haplotype ST3 is rare in steelhead populations from northern California but is common in coastal populations from the San Francisco Bay area and dominant in resident populations from the upper Sacramento River and the Kern and Little Kern rivers (Nielsen et al. 1997*b*, 1998; Bagley and Gall 1998). Haplotypes ST1 and ST3 were found in equal frequencies in steelhead populations from central California (Table 1) (Nielsen 1996). Haplotype ST9 is rare (<2%) in coastal populations from California (J. Nielsen, US Geological Survey, Alaska Biological Science Center, 1011 East Tudor Road, Anchorage, AK 99503, USA, personal communication), but it is more common in inland steelhead populations from the Snake River in Idaho (Kucera and Armstrong 2001) and in inland populations from the upper Columbia River in Canada (McCusker et al. 2000).

To explore the hypothesis that a monomorphic sample of Santa Cruz wild fish could have originated through haplotype loss and sampling bias, as opposed to a truly monomorphic origin in the McCloud River, we applied our probabilistic model to two extreme alternative scenarios: a central California type parental stock, with a minimum 40% frequency of the ST1 haplotype, and a northern California type stock, with a maximum of 83% frequency of ST1 (Figs. 2*a* and 2*b*, respectively). As expected from first principles of a binomial sampling process, the probability of haplotype fixation increases as the number of donor females and founding fish decreases (lower left in Figs. 2*a* and 2*b*). When a central California type parental stock is considered, the probability of an all-ST1 sample remains very low (<1%) for practically all values of donor females and founding fish considered, indicating that it is highly unlikely that Santa Cruz River fish originated from such a stock (Fig. 2*a*).

**Fig. 2.** Probability isoclines of obtaining an all-ST1 sample of 20 individuals from polymorphic parental populations as a function of donor females and number of offspring colonizing the river. Frequency of the ST1 haplotype in the maternal population: (*a*) $p = 0.4$ and (*b*) $p = 0.83$. The shaded area indicates the most likely range of fecundity for Californian hatchery fish.




When a northern California type parental stock is considered, results are less clearcut, with probabilities ranging between 3% and 45% depending on the values chosen for

average fecundity and initial survival (Fig. 2*b*). This led us to scrutinize these parameters in more detail. Individual rainbow trout can have fecundities as low as 500 and as high as 13 000 eggs (Carlander 1969). We used a range of 500–4500 to accommodate probable values for individual mothers of Santa Cruz river fish, but average fecundities reported for Californian wild populations are closer to the lower half of this range. For example, Hallock et al. (1961) reported a mean number of 2808 eggs for larger Sacramento River stripped females. Perhaps more relevant to our case, Wales (1939) reported that female rainbow trout trapped at Greens Creek, the trapping site of the first egg-taking station of rainbow trout on the McCloud River, weighed 2 lb on average, with a mean fecundity between 1000 and 2000. Average fecundities lower than 2500 (at least 30 donor females) result in a probability of sampling only ST1 fish of less than 10% (Fig. 2), unless the survival from eggs to founding fish was very low (<0.00033 or <25 founding fish), in which case this probability becomes greater.

In summary, unless a small number of particularly large females (<17) had been used to produce the eggs shipped to the Santa Cruz River and (or) a very small proportion of the imported eggs survived to become founding fish (<25 individuals), the probability of obtaining an all-ST1 sample of 20 individuals from a northern California type parental stock is less than 10% (Fig. 2).

## Discussion

Our analyses allowed us to establish the most likely origin of Santa Cruz River main-stem fish as well as to advance our general knowledge on the relative contribution of different parental stocks to rainbow trout in Patagonia. As previously suggested by microsatellite analyses (Pascual et al. 2001), mtDNA data reinforce the idea that anadromous and nonanadromous Santa Cruz fish do not constitute independent lineages but have a common ancestry. Wild fish are clearly differentiated from hatchery Danish stocks widely propagated in the region after 1950, providing strong evidence for an origin of Santa Cruz populations in Californian rainbow trout imported to Argentina during the first decade of the twentieth century. Additionally, these results indicate that the introgression from hatchery fish into the wild population has not been significant.

Although it has been widely accepted that early transplants of rainbow trout from the United States to locations around the world, including Argentina, came from the McCloud River (Scott et al. 1978; Busack and Gall 1980; Pascual et al. 2002*a*), historical records alone were insufficient to verify this, conferring some credence to the idea that other locations in northern California and southern Oregon could have potentially contributed fish to these early transplants (Behnke 2002). The fact that Santa Cruz River wild fish analyzed were monomorphic for haplotype ST1, together with the results from our probabilistic model, provides additional support to the view that the source of these populations was indeed the McCloud River.

It must be noted, however, that our approach does not consider some complex scenarios that could muddle the identification of parental stocks. First, Santa Cruz fish could have been derived from a mixture of fish from the McCloud and other northern California locations, leading to a larger probability of haplotype fixation than purported by our scenarios. Second, the founding population could have experienced multiple bottleneck events instead of the single event at the onset of the introduction that we modeled, increasing as well the probability of haplotype fixation. Since there are no conceivable bounds on the exercise of conjecturing combinations of parental stocks or bottleneck sequences, we did not attempt additional analyses, leaving it simply as a precautionary note.

The clear differences found between wild and hatchery Santa Cruz fish point at mtDNA analysis as a powerful tool to elucidate the ancestral genetic makeup of rainbow trout in Patagonia at a regional scale and to determine the relative contribution of stocks used before and after 1950. The occurrence of ST3 in hatchery stocks may suggest a Californian origin, most likely a genetic heritage derived from the Sacramento River rainbow trout, while haplotype ST9, which is rare in Californian wild stocks, hints at a complex ancestry of stocks imported from Europe, with probable contributions of non-Californian fish (e.g., British Columbia, where ST9 is more frequent). However, we analyzed only a handful of hatchery fish and larger samples from different hatchery stocks will be required to fully characterize the genetic makeup of this stock.

At present, these data make no suggestion as to what extent anadromy and residency in the Santa Cruz River are merely recreating the preexistent variation or have been modified in response to the specific selective pressures of the novel environment. Nevertheless, the identification of the genetic roots of Santa Cruz fish provides relevant background information to guide future research about the origin of life history variation in this river. Regardless of the specific processes underlying life history variation, our results indicate that the Santa Cruz River may well constitute a unique, secluded reservoir of those ancestral McCloud fish widely distributed around the world during the late nineteenth and early twentieth centuries, which in their native range have been substantially affected by habitat modification and introgression from hatchery stocks (Busby et al. 1996; Nielsen et al. 1999; McEwan 2001).

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