# CHAPTER III
# RESULTS

## *Genetic Diversity*

Genotypes at 13 microsatellite DNA loci were determined for 238 brook trout sampled from 22 streams, representing 4 major river drainages in New Jersey, and 1 trout hatchery (see Table 1 for listing and abbreviations; Figure 1). The allele frequencies, individual locus heterozygosities, overall mean heterozygosities, and mean number of alleles per locus are provided in Table A1.2, Appendix. A total of 136 alleles was detected in 23 collections and the number of alleles per locus ranged from 2 (*SfoC79*) to 24 (*SfoC115*), with a mean of 10.5. When the hatchery collection excluded, the total number of alleles per locus was 133 (mean 10.2). Allelic richness was lowest in the Mason's Run collection from south Jersey (MAS, 1.7) and greatest in the hatchery collection (PTH, 4.7) (Table 3).

Observed heterozygosity ($H_o$) across the 23 collections averaged 0.541, and in a majority of collections (15 or 65%), ranged from 0.500 to 0.700 (Table 3). $H_o$ was lowest in animals from Masons Run (MAS; 0.342), and highest in animals from Cooley's Brook (COO, 0.734), a stream having a history of trout stocking. The hatchery collection had

TABLE 3.—Summary of genetic diversity statistics for 23 collections of brook trout (*Salvelinus fontinalis*) from New Jersey surveyed at 13 microsatellite loci. Sample size (N), mean number of alleles per locus ($\hat{A}$ ), allelic richness ($\hat{A}_C$ corrected to *n*=7 for collections and *n*=9 for drainages), number of private alleles, polymorphism (frequency of most common allele did not exceed 0.95), observed and expected heterozygosity, and estimates of the inbreeding coefficient ($F_{IS}$ ).

| Drainage (or hatchery) of origin | Collection | | | | Private | | Percent of loci | Heterozygosity | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Stream of origin | abbreviation | N | $\hat{A}$ | $\hat{A}_C$ | alleles[a] | | polymorphic | ( $H_O$ ) | ( $H_E$ ) | $F_{IS}$ |
| ***Delaware drainage*** | | **55** | **7.7** | | **11** | **(12)** | **100.0** | **0.534** | **0.685** | **0.222** |
| Forked Brook | FOR | 9 | 4.3 | 4.0 | 2 | (3) | 92.3 | 0.615 | 0.619 | 0.007 |
| Van Campens Brook [b] | VCB | 9 | 4.5 | 4.1 | 2 | (2) | 92.3 | 0.547 | 0.608 | 0.106 |
| Independence Brook | IND | 11 | 3.6 | 3.2 | 0 | (0) | 92.3 | 0.580 | 0.521 | -0.121 |
| Halfway House Brook | HWH | 8 | 3.2 | 3.2 | 1 | (1) | 92.3 | 0.596 | 0.573 | -0.043 |
| Kurtenbach's Brook | KUR | 9 | 3.0 | 2.9 | 3 | (3) | 92.3 | 0.521 | 0.522 | 0.002 |
| Masons Run | MAS | 9 | 1.7 | 1.7 | 0 | (0) | 61.5 | 0.342 | 0.309 | -0.115 |
| ***Hudson drainage*** | | **10** | **3.5** | | **0** | **(0)** | **92.3** | **0.575** | **0.546** | **-0.058** |
| Mud Pond Outlet Stream | MPO | 10 | 3.5 | 3.3 | 0 | (0) | 92.3 | 0.575 | 0.546 | -0.058 |
| ***Passaic-Hackensack drainage*** | | **80** | **7.7** | | **10** | **(14)** | **100.0** | **0.501** | **0.668** | **0.251** |
| Cresskill Brook | CRE | 11 | 2.5 | 2.5 | 1 | (3) | 84.6 | 0.508 | 0.452 | -0.131 |
| Preakness Brook | PRE | 11 | 2.0 | 2.0 | 0 | (1) | 84.6 | 0.350 | 0.339 | -0.320 |
| Havemeyer Brook | HAV | 7 | 2.8 | 2.8 | 1 | (1) | 76.9 | 0.549 | 0.469 | -0.188 |
| Cooleys Brook [b] | COO | 11 | 4.5 | 4.1 | 0 | (0) | 100.0 | 0.734 | 0.679 | -0.086 |
| Burnt Meadow Brook | BMB | 11 | 2.9 | 2.6 | 1 | (1) | 76.9 | 0.472 | 0.432 | -0.097 |
| Lake Stockholm Brook | LSB | 10 | 2.9[c] | 2.7[c] | 0 | (0) | 91.7[c] | 0.475[c] | 0.430[c] | -0.110[c] |
| Hibernia Brook | HIB | 10 | 3.4 | 3.1 | 2 | (2) | 92.3 | 0.554 | 0.534 | -0.038 |
| Crooked Brook tributary | CBT | 9 | 2.2 | 2.2 | 1 | (1) | 84.6 | 0.353 | 0.346 | -0.020 |
| ***Raritan drainage*** | | **73** | **7.9** | | **5** | **(7)** | **100.0** | **0.549** | **0.694** | **0.211** |
| Flanders Brook [b] | FLA | 13 | 4.2 | 3.7 | 2 | (2) | 92.3 | 0.613 | 0.619 | 0.010 |
| Krueger's Creek | KRU | 10 | 4.2 | 3.9 | 0 | (0) | 100.0 | 0.623 | 0.631 | 0.013 |
| Turkey Brook | TUR | 10 | 4.9 | 4.4 | 1 | (1) | 92.3 | 0.684 | 0.652 | -0.052 |
| S. of Hoffmans tributary | SOH | 10 | 3.7 | 3.2 | 0 | (0) | 92.3 | 0.469 | 0.493 | 0.051 |
| Rocky Run | ROC | 10 | 3.0 | 2.9 | 1 | (1) | 92.3 | 0.507 | 0.523 | 0.032 |
| Oakdale Creek | OAK | 10 | 2.2 | 2.1 | 1 | (1) | 76.9 | 0.391 | 0.311 | -0.275 |
| Hacklebarney Brook [b] | HAC | 10 | 4.5 | 4.1 | 0 | (1) | 100.0 | 0.541 | 0.625 | 0.141 |
| ***Pequest Trout Hatchery*** | **PTH** | **20** | **6.1** | **4.7** | **3** | **–** | **100.0** | **0.677** | **0.695** | **0.026** |
| ***Total*** | | **238** | | | **22** | **(24)** | | | | |

[a] ( ) indicates the number of private alleles when the hatchery collection is excluded; [b] Stream has a known history of stocking; [c] value based upon 12 loci (D91 not included).

the third highest observed heterozygosity (PTH, 0.677), which was substantially higher than those found when the collections were pooled by drainage ($H_o$ ranged from 50.1 to 57.5 % in the 4 drainages). The percentage of polymorphic loci ranged from 61.5% in Mason's Run, to 100% in four collections (Cooley's Brook, Kruegers Creek, Hacklebarney Brook, and Pequest Trout Hatchery) (Table 3). Inbreeding ($F_{IS}>0$) was detected in eight collections from wild populations (0.002 – 0.141), and the hatchery collection (0.026).

Randomization tests showed that nearly all of the genotypic frequencies observed in the 23 collections conformed to Hardy–Weinberg (HW) expectations. Just by chance alone, 15 differences would be expected at the 0.05 level (0.05 x 23 x 13). A significant departure from HW proportions was detected in only 1 of 299 locus-by collection comparisons ($\alpha = 0.05$, $P < 0.002$). This departure was observed at the locus *SfoD75* in the collection from Hacklebarney Brook (*P*-value of 0.0002), and was the result of a heterozygote deficiency (homozygote excess) in 1 animal (HAC-08). A heterozygote deficit is the more common direction of HW equilibrium deviation, and can be due to the biological realities of violating the criteria of an ideal population, such as strong inbreeding or selection for or against a certain allele (Selkoe and Toonen 2006). Failure of this locus to meet Hardy–Weinberg expectations in one animal was not considered grounds for discarding the locus. Of the 1794 pairwise tests for linkage disequilibrium, no significant genetic linkage was observed between any paired loci across all collections ($\alpha = 0.05$, $P < 0.00017$), indicating the loci are segregating independently.

Heterogeneous allele frequencies were observed through the study area. Among 3,267 single-locus pairwise tests (3,289 less 22 for no genotype at locus *SfoD91* in the LSB collection) of allele frequency heterogeneity, 1283 (41.1%) exhibited departures from homogeneity after correction for multiple tests ($\alpha = 0.05$, $P < 0.00003$).

A total of 22 private alleles (16%) were found distributed in 14 of the 23 collections, at frequencies ranging from 0.025 to 0.333 (Table 4). The highest number per collection (3) was found in Kurtenbach's Brook (KUR) and the Pequest Trout Hatchery (PTH). Ten of the private alleles occurred at relatively high frequencies (at least 0.1) in 8 different streams (Kurtenbach's Brook (2), Flanders Brook (2), Halfway House Brook, Cresskill Brook, Burnt Meadow Brook, Hibernia Brook, Crooked Brook tributary, and Rocky Run). Five more private alleles were detected when the hatchery collection was excluded and one of those (in Cresskill Brook) occurred at a frequency in excess of 0.1. When the collections were pooled by drainage (with the hatchery collection considered a drainage), more private alleles (29, 21%) were found (Table 5). However, the highest frequency detected was 20.3%, and most (27, 93%) occurred at a frequency lower than 10%. When the hatchery collection was excluded from this analysis, 7 additional private alleles were detected at low frequencies (< 10%).

When the 22 collections representing spawning brook trout populations were grouped by drainage, quantitative estimates of hierarchical gene diversity indicated significant genetic diversity at every level. The greatest amount of variation occurred within populations (50.8%), followed by variation among populations within drainages (27.5%), and variation among drainages (21.7%) (Figure 11A). A comparison between the four drainages, with all collections pooled by drainage, determined that 73.4% of the genetic variation was due to differentiation within drainages and only 26.6% of the variation occurred between drainages (Figure 11B).

TABLE 4.— Frequency of 21 unique alleles found in 238 brook trout collected from 22 streams and a hatchery in New Jersey, surveyed at 13 microsatellite loci. Additional unique alleles (those with corresponding frequencies shown in parentheses) were detected when the Pequest Trout Hatchery collection was excluded from analysis.

| Drainage (or hatchery) of origin<br>Stream of origin | Collection abbreviation | Locus | Allele size | Frequency |
|---|---|---|---|---|
| ***Delaware drainage*** | | | | |
| Forked Brook | FOR | *Sfo-C28* | 177 | 0.056 |
| | FOR | *Sfo-D91* | 256 | ( 0.050 ) |
| | FOR | *Sfo-D100* | 282 | 0.056 |
| Van Campens Brook [a] | VCB | *Sfo-C113* | 124 | 0.056 |
| | VCB | *Sfo-C115* | 329 | 0.056 |
| Halfway House Brook | HWH | *Sfo-D100* | 258 | 0.125 |
| Kurtenbach's Brook | KUR | *Sfo-D75* | 216 | 0.222 |
| | KUR | *Sfo-D100* | 242 | 0.333 |
| | KUR | *Sfo-D100* | 270 | 0.056 |
| ***Hudson drainage*** | | - | - | - |
| ***Passaic-Hackensack drainage*** | | | | |
| Cresskill Brook | CRE | *Sfo-C86* | 122 | 0.227 |
| | CRE | *Sfo-C115* | 333 | ( 0.075 ) |
| | CRE | *Sfo-D75* | 192 | ( 0.125 ) |
| Preakness Brook | PRE | *Sfo-B52* | 227 | ( 0.050 ) |
| Havemeyer Brook | HAV | *Sfo-C115* | 343 | 0.071 |
| Burnt Meadow Brook | BMB | *Sfo-C113* | 157 | 0.273 |
| Hibernia Brook | HIB | *Sfo-C86* | 128 | 0.200 |
| | HIB | *Sfo-C115* | 357 | 0.050 |
| Crooked Brook tributary | CBT | *Sfo-C28* | 203 | 0.333 |
| ***Raritan drainage*** | | | | |
| Flanders Brook [a] | FLA | *Sfo-C24* | 125 | 0.154 |
| | FLA | *Sfo-C115* | 303 | 0.269 |
| Turkey Brook | TUR | *Sfo-C115* | 321 | 0.056 |
| Rocky Run | ROC | *Sfo-C113* | 136 | 0.111 |
| Oakdale Creek | OAK | *Sfo-C28* | 197 | 0.050 |
| Hacklebarney Brook [a] | HAC | *Sfo-B52* | 199 | ( 0.025 ) |
| ***Pequest Trout Hatchery*** | PTH | *Sfo-B52* | 207 | 0.050 |
| | PTH | *Sfo-C28* | 199 | 0.025 |
| | PTH | *Sfo-D91* | 280 | 0.075 |

[b] Stream has a known history of stocking.

TABLE 5.— Frequency of 29 unique alleles found in 238 brook trout collected from 4 drainages and a hatchery in New Jersey, surveyed at 13 microsatellite loci. Additional unique alleles (those with corresponding frequencies shown in parentheses) were detected when the Pequest Trout Hatchery collection was excluded from analysis.

| Drainage or hatchery of origin | Locus | Allele size | Frequency |
|---|---|---|---|
| Delaware drainage | *Sfo-C28* | 177 | 0.009 |
| | *Sfo-C113* | 124 | 0.009 |
| | *Sfo-C115* | 329 | 0.009 |
| | *Sfo-D75* | 216 | 0.036 |
| | *Sfo-D91* | 244 | 0.036 |
| | *Sfo-D91* | 252 | 0.018 |
| | *Sfo-D91* | 256 | ( 0.050 ) |
| | *Sfo-D100* | 242 | 0.055 |
| | *Sfo-D100* | 258 | 0.018 |
| | *Sfo-D100* | 270 | 0.009 |
| | *Sfo-D100* | 274 | 0.027 |
| | *Sfo-D100* | 282 | 0.009 |
| Hudson drainage | - | - | - |
| Passaic-Hackensack drainage | *Sfo-B52* | 227 | ( 0.050 ) |
| | *Sfo-C28* | 195 | 0.019 |
| | *Sfo-C28* | 203 | 0.038 |
| | *Sfo-C86* | 122 | 0.031 |
| | *Sfo-C86* | 128 | 0.025 |
| | *Sfo-C113* | 157 | 0.038 |
| | *Sfo-C115* | 249 | ( 0.025 ) |
| | *Sfo-C115* | 333 | ( 0.075 ) |
| | *Sfo-C115* | 343 | 0.006 |
| | *Sfo-C115* | 345 | 0.203 |
| | *Sfo-C115* | 349 | 0.057 |
| | *Sfo-C115* | 353 | 0.152 |
| | *Sfo-C115* | 357 | 0.006 |
| | *Sfo-D75* | 192 | ( 0.125 ) |
| Raritan drainage | *Sfo-B52* | 199 | ( 0.025 ) |
| | *Sfo-C24* | 125 | 0.028 |
| | *Sfo-C28* | 197 | 0.007 |
| | *Sfo-C113* | 136 | 0.014 |
| | *Sfo-C115* | 303 | 0.049 |
| | *Sfo-C115* | 321 | 0.007 |
| | *Sfo-C129* | 236 | ( 0.050 ) |
| Pequest Trout Hatchery | *Sfo-B52* | 207 | 0.050 |
| | *Sfo-C28* | 199 | 0.025 |
| | *Sfo-D91* | 280 | 0.075 |

FIGURE 11.— Hierarchical gene diversity analysis (AMOVA) of 22 spawning populations of brook trout from New Jersey for 12 microsatellite DNA loci ($P < 0.010$). (A) Populations grouped by drainage but not pooled; (B) Populations pooled by drainage.

A considerable amount of genetic differentiation was also observed in comparisons of $F_{ST}$ values. Pairwise $F_{ST}$ estimates ranged from 0.07 between Cooley's Brook (COO), a stream having a history of trout stocking, and Pequest Trout Hatchery (PTH) collections, to 0.602 between collections from different drainages (Preakness Brook, PRE and Masons Run, MAS) (Table 6, below diagonal). Of the 253 comparisons, 250 (99%) were greater than 0.100, and 189 (75%) were greater than 0.200. $R_{ST}$ values also indicated similar differences between pairs of collections, with values ranging from -0.001 to 0.935 (Table 6, above diagonal).

### ***Population Structure***

Pairwise genetic distance values (chord distance values; Cavelli-Sforza and Edwards 1967) were calculated between all collections to investigate evolutionary relationships among allele frequencies (Table 7). The greatest genetic distance occurred between two collections from different drainages (Preakness Brook, PRE and Rocky Run, ROC; 0.817), and 13 of 14 pairs having the greatest genetic distance involved the Preakness Brook collection. The lowest genetic distance was observed between the Pequest Trout Hatchery collection (PTH) and the collection from Cooleys Brook (COO), a stream having a history of trout stocking. The unrooted neighbor-joining tree depicting the underlying genetic structure of the distance matrix illustrates differentiation among collections by drainage (Figure 12). Two distinct groups were formed which were comprised of populations representing the Raritan and Passaic-Hackensack drainages. The Raritan drainage grouping contained 6 of 7 collections originating from the drainage,

TABLE 6.— Matrix of $F_{ST}$ values (below the diagonal) and $R_{ST}$ values (above the diagonal) for all pairwise comparisons among 23 brook trout collections from New Jersey. Measures were derived from data for 13 microsatellite loci. See Table 1 for collection abbreviations.

| Collection | FOR | VCB | IND | HWH | KUR | MAS | MPO | CRE | PRE | HAV | COO | BMB | LSO | HIB | CBT | FLA | KRU | TUR | SOH | ROC | OAK | HAC | PTH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FOR |  | 0.251 | 0.065 | 0.387 | 0.145 | 0.805 | 0.380 | 0.870 | 0.930 | 0.525 | 0.244 | 0.756 | 0.619 | 0.516 | 0.880 | 0.522 | 0.387 | 0.225 | 0.258 | 0.250 | 0.577 | 0.242 | 0.321 |
| VCB | 0.091 |  | 0.079 | 0.017 | 0.278 | 0.339 | 0.079 | 0.577 | 0.760 | 0.160 | 0.084 | 0.460 | 0.180 | 0.178 | 0.655 | 0.111 | 0.050 | 0.040 | 0.031 | 0.256 | 0.147 | -0.001 | 0.041 |
| IND | 0.120 | 0.203 |  | 0.194 | 0.194 | 0.561 | 0.168 | 0.705 | 0.833 | 0.328 | 0.153 | 0.622 | 0.377 | 0.382 | 0.758 | 0.271 | 0.164 | 0.093 | 0.074 | 0.079 | 0.363 | 0.112 | 0.160 |
| HWH | 0.189 | 0.244 | 0.266 |  | 0.372 | 0.328 | 0.129 | 0.575 | 0.754 | 0.158 | 0.085 | 0.428 | 0.206 | 0.126 | 0.632 | 0.171 | 0.096 | 0.080 | 0.076 | 0.379 | 0.174 | 0.043 | 0.063 |
| KUR | 0.200 | 0.279 | 0.315 | 0.251 |  | 0.795 | 0.404 | 0.881 | 0.932 | 0.533 | 0.151 | 0.745 | 0.682 | 0.498 | 0.885 | 0.568 | 0.466 | 0.193 | 0.318 | 0.409 | 0.603 | 0.267 | 0.328 |
| MAS | 0.283 | 0.358 | 0.382 | 0.364 | 0.464 |  | 0.355 | 0.743 | 0.901 | 0.216 | 0.445 | 0.298 | 0.468 | 0.083 | 0.781 | 0.327 | 0.415 | 0.429 | 0.570 | 0.822 | 0.385 | 0.422 | 0.161 |
| MPO | 0.178 | 0.270 | 0.287 | 0.167 | 0.310 | 0.337 |  | 0.610 | 0.790 | 0.207 | 0.119 | 0.497 | 0.257 | 0.235 | 0.690 | 0.082 | 0.090 | 0.189 | 0.219 | 0.417 | 0.217 | 0.099 | -0.028 |
| CRE | 0.236 | 0.275 | 0.213 | 0.276 | 0.389 | 0.399 | 0.291 |  | 0.732 | 0.316 | 0.699 | 0.316 | 0.452 | 0.216 | 0.406 | 0.508 | 0.531 | 0.656 | 0.707 | 0.866 | 0.571 | 0.661 | 0.450 |
| PRE | 0.396 | 0.349 | 0.462 | 0.421 | 0.456 | 0.602 | 0.457 | 0.527 |  | 0.573 | 0.812 | 0.338 | 0.755 | 0.416 | 0.419 | 0.773 | 0.765 | 0.786 | 0.840 | 0.935 | 0.782 | 0.808 | 0.660 |
| HAV | 0.198 | 0.224 | 0.263 | 0.302 | 0.312 | 0.444 | 0.333 | 0.365 | 0.355 |  | 0.328 | 0.187 | 0.086 | 0.061 | 0.398 | 0.112 | 0.144 | 0.206 | 0.275 | 0.464 | 0.162 | 0.244 | 0.143 |
| COO | 0.122 | 0.156 | 0.192 | 0.145 | 0.264 | 0.298 | 0.138 | 0.259 | 0.373 | 0.260 |  | 0.574 | 0.421 | 0.291 | 0.738 | 0.309 | 0.220 | 0.098 | 0.137 | 0.345 | 0.323 | 0.061 | 0.110 |
| BMB | 0.234 | 0.272 | 0.346 | 0.340 | 0.323 | 0.452 | 0.331 | 0.385 | 0.378 | 0.250 | 0.277 |  | 0.357 | 0.143 | 0.306 | 0.441 | 0.482 | 0.513 | 0.592 | 0.747 | 0.427 | 0.533 | 0.394 |
| LSB | 0.153 | 0.190 | 0.207 | 0.262 | 0.368 | 0.451 | 0.292 | 0.331 | 0.426 | 0.214 | 0.197 | 0.300 |  | 0.144 | 0.582 | 0.097 | 0.088 | 0.329 | 0.322 | 0.595 | 0.139 | 0.268 | 0.169 |
| HIB | 0.175 | 0.245 | 0.216 | 0.199 | 0.319 | 0.368 | 0.226 | 0.234 | 0.439 | 0.305 | 0.188 | 0.336 | 0.281 |  | 0.289 | 0.172 | 0.178 | 0.249 | 0.307 | 0.510 | 0.096 | 0.226 | 0.177 |
| CBT | 0.270 | 0.350 | 0.355 | 0.337 | 0.452 | 0.483 | 0.333 | 0.414 | 0.524 | 0.380 | 0.281 | 0.330 | 0.315 | 0.286 |  | 0.653 | 0.633 | 0.698 | 0.748 | 0.879 | 0.658 | 0.718 | 0.572 |
| FLA | 0.132 | 0.171 | 0.196 | 0.237 | 0.251 | 0.341 | 0.245 | 0.270 | 0.400 | 0.215 | 0.200 | 0.277 | 0.194 | 0.213 | 0.299 |  | 0.040 | 0.253 | 0.290 | 0.499 | 0.122 | 0.173 | 0.024 |
| KRU | 0.170 | 0.170 | 0.232 | 0.172 | 0.266 | 0.397 | 0.220 | 0.287 | 0.318 | 0.214 | 0.170 | 0.240 | 0.195 | 0.196 | 0.307 | 0.184 |  | 0.110 | 0.141 | 0.370 | 0.101 | 0.083 | 0.052 |
| TUR | 0.122 | 0.176 | 0.215 | 0.185 | 0.195 | 0.322 | 0.209 | 0.283 | 0.330 | 0.163 | 0.185 | 0.256 | 0.237 | 0.169 | 0.306 | 0.132 | 0.177 |  | 0.041 | 0.161 | 0.289 | 0.069 | 0.149 |
| SOH | 0.188 | 0.232 | 0.227 | 0.293 | 0.320 | 0.469 | 0.322 | 0.352 | 0.464 | 0.256 | 0.218 | 0.344 | 0.173 | 0.297 | 0.394 | 0.188 | 0.206 | 0.189 |  | 0.200 | 0.343 | 0.094 | 0.173 |
| ROC | 0.214 | 0.270 | 0.276 | 0.242 | 0.298 | 0.398 | 0.276 | 0.308 | 0.520 | 0.261 | 0.233 | 0.380 | 0.292 | 0.276 | 0.417 | 0.228 | 0.284 | 0.212 | 0.279 |  | 0.604 | 0.311 | 0.337 |
| OAK | 0.276 | 0.366 | 0.262 | 0.414 | 0.428 | 0.543 | 0.415 | 0.439 | 0.580 | 0.416 | 0.336 | 0.481 | 0.312 | 0.374 | 0.484 | 0.326 | 0.351 | 0.336 | 0.335 | 0.435 |  | 0.149 | 0.100 |
| HAC | 0.127 | 0.184 | 0.172 | 0.226 | 0.252 | 0.345 | 0.258 | 0.291 | 0.420 | 0.242 | 0.165 | 0.327 | 0.184 | 0.244 | 0.343 | 0.142 | 0.189 | 0.137 | 0.140 | 0.236 | 0.205 |  | 0.053 |
| PTH | 0.110 | 0.154 | 0.188 | 0.110 | 0.225 | 0.259 | 0.092 | 0.217 | 0.324 | 0.238 | 0.072 | 0.239 | 0.191 | 0.173 | 0.271 | 0.177 | 0.142 | 0.137 | 0.209 | 0.189 | 0.313 | 0.145 |  |

TABLE 7.─Genetic distance (chord distance, Cavalli-Sforza and Edwards 1967) among 23 collections of brook trout from New Jersey using 13 microsatellite loci. See Table 1 for collection abbreviations.

| Collection | FOR | VCB | IND | HWH | KUR | MAS | MPO | CRE | PRE | HAV | COO | BMB | LSB | HIB | CBT | FLA | KRU | TUR | SOH | ROC | OAK | HAC | PTH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FOR | | | | | | | | | | | | | | | | | | | | | | | |
| VCB | 0.455 | | | | | | | | | | | | | | | | | | | | | | |
| IND | 0.451 | 0.527 | | | | | | | | | | | | | | | | | | | | | |
| HWH | 0.576 | 0.606 | 0.604 | | | | | | | | | | | | | | | | | | | | |
| KUR | 0.515 | 0.619 | 0.581 | 0.591 | | | | | | | | | | | | | | | | | | | |
| MAS | 0.565 | 0.614 | 0.606 | 0.606 | 0.676 | | | | | | | | | | | | | | | | | | |
| MPO | 0.549 | 0.615 | 0.635 | 0.541 | 0.669 | 0.592 | | | | | | | | | | | | | | | | | |
| CRE | 0.575 | 0.608 | 0.498 | 0.604 | 0.668 | 0.603 | 0.625 | | | | | | | | | | | | | | | | |
| PRE | 0.708 | 0.651 | 0.738 | 0.687 | 0.707 | 0.751 | 0.715 | 0.762 | | | | | | | | | | | | | | | |
| HAV | 0.542 | 0.579 | 0.557 | 0.600 | 0.620 | 0.634 | 0.672 | 0.631 | 0.569 | | | | | | | | | | | | | | |
| COO | 0.523 | 0.531 | 0.549 | 0.539 | 0.649 | 0.582 | 0.490 | 0.610 | 0.687 | 0.619 | | | | | | | | | | | | | |
| BMB | 0.590 | 0.613 | 0.634 | 0.663 | 0.601 | 0.637 | 0.637 | 0.655 | 0.571 | 0.531 | 0.583 | | | | | | | | | | | | |
| LSB | 0.473 | 0.477 | 0.481 | 0.493 | 0.631 | 0.616 | 0.562 | 0.588 | 0.619 | 0.460 | 0.491 | 0.536 | | | | | | | | | | | |
| HIB | 0.533 | 0.568 | 0.540 | 0.571 | 0.633 | 0.615 | 0.613 | 0.552 | 0.679 | 0.590 | 0.561 | 0.654 | 0.534 | | | | | | | | | | |
| CBT | 0.587 | 0.612 | 0.613 | 0.617 | 0.712 | 0.607 | 0.604 | 0.649 | 0.658 | 0.580 | 0.611 | 0.561 | 0.496 | 0.560 | | | | | | | | | |
| FLA | 0.507 | 0.543 | 0.543 | 0.604 | 0.598 | 0.610 | 0.614 | 0.617 | 0.724 | 0.568 | 0.609 | 0.633 | 0.516 | 0.576 | 0.619 | | | | | | | | |
| KRU | 0.540 | 0.543 | 0.563 | 0.533 | 0.641 | 0.670 | 0.574 | 0.633 | 0.622 | 0.546 | 0.543 | 0.579 | 0.459 | 0.530 | 0.578 | 0.511 | | | | | | | |
| TUR | 0.487 | 0.540 | 0.525 | 0.550 | 0.535 | 0.600 | 0.572 | 0.628 | 0.624 | 0.496 | 0.588 | 0.593 | 0.513 | 0.528 | 0.593 | 0.501 | 0.448 | | | | | | |
| SOH | 0.539 | 0.568 | 0.542 | 0.579 | 0.631 | 0.677 | 0.603 | 0.645 | 0.708 | 0.548 | 0.556 | 0.599 | 0.449 | 0.575 | 0.622 | 0.530 | 0.487 | 0.492 | | | | | |
| ROC | 0.551 | 0.629 | 0.543 | 0.573 | 0.645 | 0.634 | 0.616 | 0.586 | 0.817 | 0.548 | 0.597 | 0.666 | 0.566 | 0.601 | 0.644 | 0.580 | 0.612 | 0.556 | 0.552 | | | | |
| OAK | 0.545 | 0.621 | 0.518 | 0.667 | 0.627 | 0.655 | 0.661 | 0.663 | 0.735 | 0.625 | 0.644 | 0.681 | 0.530 | 0.591 | 0.623 | 0.627 | 0.610 | 0.599 | 0.549 | 0.652 | | | |
| HAC | 0.467 | 0.524 | 0.487 | 0.569 | 0.600 | 0.587 | 0.609 | 0.635 | 0.721 | 0.593 | 0.529 | 0.656 | 0.488 | 0.575 | 0.615 | 0.500 | 0.528 | 0.487 | 0.472 | 0.569 | 0.467 | | |
| PTH | 0.495 | 0.520 | 0.542 | 0.500 | 0.632 | 0.597 | 0.429 | 0.572 | 0.654 | 0.617 | 0.377 | 0.607 | 0.493 | 0.561 | 0.613 | 0.567 | 0.516 | 0.535 | 0.563 | 0.587 | 0.638 | 0.489 | |

including 2 streams having a history of trout stocking (Flanders Brook, FLA and Hacklebarney Brook, HAC). One collection from the Raritan drainage, Rocky Run (ROC), was left out of this genetically related group and one collection from the Passaic-Hackensack drainage, Lake Stockholm Brook (LSB), also clustered with this group. In the Passaic-Hackensack drainage, 5 of 8 collections also formed a group. One of the collections from the Passaic-Hackensack drainage not included in this subgroup was Cresskill Brook (CRE), the only collection from the Hackensack River watershed which drains into the Newark Bay complex and not directly into the Passaic River. The six collections from the Delaware drainage, which generally are more isolated from each other compared to collections within other drainages, separated into two smaller groups. The phenogram also illustrates a high level of divergence associated with the Pequest Trout Hatchery (PTH) collection, the only collection known to be comprised of brook trout not native to New Jersey. Animals from this collection were most closely related to animals from Cooley's Brook (COO), a stream having a long history of stocking whose collection did not group with others originating from the same drainage (Passaic-Hackensack). The Hudson drainage, represented by only one collection (Mud Pond Outlet Stream, MPO), was highly differentiated from the two major groupings (Raritan and Passaic-Hackensack ) and closely related to the collections from Cooley's Brook and the Pequest Trout Hatchery.

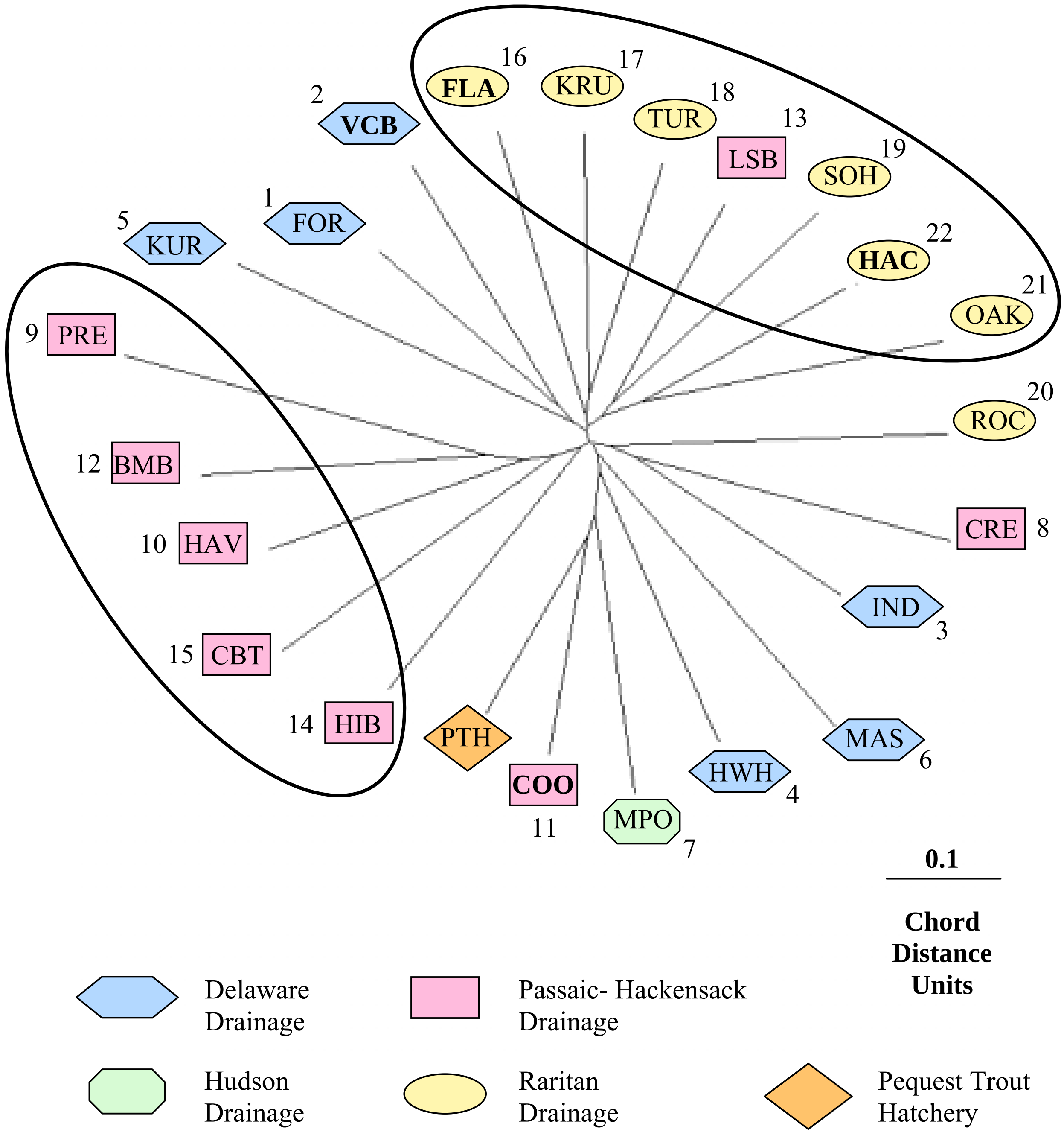

FIGURE 12.— Neighbor-joining phenogram constructed from the genetic distance matrix using the chord distance of Cavelli-Sforza and Edwards (1967), for brook trout collected from 22 streams and 1 hatchery in New Jersey. Collections in bold are from streams having a known history of brook trout stocking (see Table 1, Figure 7 for collection abbreviations and locations, and Table 7 for distance values).

### *Individual Assignment*

Individual assignment tests using multilocus genotypes revealed that population differentiation was sufficient to identify the origin of individual fish with a high rate of success. Individuals were correctly assigned to their population of origin 94.5% of the time (on average) across all populations (Table 8). Fifteen populations had a 100% assignment success rate, and the remaining 8 populations had a total of 13 fish incorrectly assigned. There was no apparent pattern to the incorrect assignments, even within the 4 populations that had multiple fish (2 or 3) incorrectly assigned. When the populations were pooled by drainage, with the hatchery population considered a drainage, the assignment success dropped to 87.0% (207/238 fish assigned to the correct drainage) (Table 9).

TABLE 8.—Results of maximum likelihood assignment tests for 23 brook trout collections from New Jersey using multilocus genotypes derived from 12 microsatellite DNA markers. See Table 1 for collection abbreviations (collections in bold are from streams having a known history of brook trout stocking). Each row shows the sample size (*N*) and the assignment of individuals from the specified collection to all collections. The values along the diagonal (bold italics) indicate the number of correct assignments to each collection. Boxes highlight the assignment of individuals to collections within a drainage (*n* is the number of individuals collected from the drainage). The final column indicates the rate of correct assignment of individuals to their collection. Overall, of the 238 individuals collected, 94.5% (225) were assigned to their collection of origin.

| Collection | *N* | FOR | **VCB** | IND | HWH | KUR | MAS | MPO | CRE | PRE | HAV | **COO** | BMB | LSB | HIB | CBT | **FLA** | KRU | TUR | SOH | ROC | OAK | **HAC** | PTH | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FOR | 9 | ***8*** | | 1 | | | | | | | | | | | | | | | | | | | | | 88.9 |
| **VCB** | 9 | | ***8*** | | 1 | | | | | | | | | | | | | | | | | | | | 88.9 |
| IND | 11 | | | ***11*** | | | | | | | | | | | | | | | | | | | | | 100.0 |
| HWH | 8 | | | | ***8*** | | | | | | | | | | | | | | | | | | | | 100.0 |
| KUR | 9 | | | | | ***9*** | | | | | | | | | | | | | | | | | | | 100.0 |
| MAS | 9 | | | | | | ***9*** | | | | | | | | | | | | | | | | | | 100.0 |
| MPO | 10 | | | | | | | ***10*** | | | | | | | | | | | | | | | | | 100.0 |
| CRE | 11 | | | | | | | | ***11*** | | | | | | | | | | | | | | | | 100.0 |
| PRE | 11 | | | | | | | | | ***11*** | | | | | | | | | | | | | | | 100.0 |
| HAV | 7 | | | | | | | | | | ***7*** | | | | | | | | | | | | | | 100.0 |
| **COO** | 11 | | | | | | | | | | | ***10*** | | | | | | | | | | | | 1 | 90.9 |
| BMB | 11 | | | | | | | | | | | | ***11*** | | | | | | | | | | | | 100.0 |
| LSB | 10 | | | | | | | | | | | | | ***10*** | | | | | | | | | | | 100.0 |
| HIB | 10 | | | | | | | | | | | | | | ***7*** | | | | | 1 | | 1 | | 1 | 70.0 |
| CBT | 9 | | | | | | | | | | | | | | | ***9*** | | | | | | | | | 100.0 |
| **FLA** | 13 | | | | | | | | | | | | | | | | ***13*** | | | | | | | | 100.0 |
| KRU | 10 | | | | | | | | | | | | | | | | | ***8*** | 1 | 1 | | | | | 80.0 |
| TUR | 10 | | | 1 | | | | | | | | | | | 1 | | | | ***8*** | | | | | | 80.0 |
| SOH | 10 | | | | | | | | | | | | | | 1 | | | | | ***9*** | | | | | 90.0 |
| ROC | 10 | | | | | | | | | | | | | | | | | | | | ***10*** | | | | 100.0 |
| OAK | 10 | | | | | | | | | | | | | | | | | | | | | ***10*** | | | 100.0 |
| **HAC** | 10 | | | | | | | | | | | | | | | | | | | | | | ***10*** | | 100.0 |
| PTH | 20 | | | | | | | 1 | | | | 1 | | | | | | | | | | | | ***18*** | 90.0 |

Delaware drainage *n* = 57

Hudson drainage *n* = 10

Passaic-Hackensack drainage *n* = 80

Raritan drainage *n* = 73

Hatchery *n* = 20

TABLE 9.—Results of maximum likelihood assignment tests for 23 brook trout collections from four New Jersey drainages and a hatchery using multilocus genotypes derived from 12 microsatellite DNA markers. See Table 1 for collection abbreviations (collections in bold are from streams having a known history of brook trout stocking). Each row shows the sample size (*N*) and the assignment of individuals from the specified collection to each drainage. Boxes highlight the assignment of individuals to their drainage. The final column indicates the rate of correct assignment of individuals to their drainage. Overall, of the 238 individuals collected, 87.0% (207) were assigned to the correct drainage.

| | | Drainage | | | | | |
|---|---|---|---|---|---|---|---|
| Collection | *N* | Delaware | Wallkill | Passaic - Hackensack | Raritan | Pequest Trout Hatchery | % |
| FOR | 9 | 7 | | | 2 | | 77.8 |
| **VCB** | 9 | 6 | | 2 | | 1 | 66.7 |
| IND | 11 | 11 | | | | | 100.0 |
| HWH | 8 | 6 | | | | 2 | 75.0 |
| KUR | 9 | 9 | | | | | 100.0 |
| MAS | 9 | 9 | | | | | 100.0 |
| MPO | 10 | | 10 | | | | 100.0 |
| CRE | 11 | | | 11 | | | 100.0 |
| PRE | 11 | | | 11 | | | 100.0 |
| HAV | 7 | 1 | | 6 | | | 85.7 |
| **COO** | 11 | 1 | | 5 | | 5 | 45.4 |
| BMB | 11 | 1 | | 10 | | | 90.9 |
| LSB | 10 | 3 | | 5 | 2 | | 50.0 |
| HIB | 10 | 2 | | 8 | | | 80.0 |
| CBT | 9 | | | 9 | | | 100.0 |
| **FLA** | 13 | | | | 13 | | 100.0 |
| KRU | 10 | | | 4 | 6 | | 60.0 |
| TUR | 10 | 1 | | | 9 | | 90.0 |
| SOH | 10 | | | 1 | 9 | | 90.0 |
| ROC | 10 | 1 | | | 9 | | 90.0 |
| OAK | 10 | | | | 10 | | 100.0 |
| **HAC** | 10 | | | | 9 | 1 | 90.0 |
| PTH | 20 | | 1 | | | 19 | 95.0 |