Table S1. Primer sequence, repeat motif, fluorescent label, multiplex pool and amplicon size for the monomorphic *Ambystoma maculatum* locus Am\_60.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Locus** | **Primer sequence 5' to 3'** | **Motif** | **Label** | **Multiplex** | **Size** |
| *Am\_60* | F: ATGTGTATCACCCACCGTGC | AAAC | NED | 2 | 84–84 |
|  | R: TCTGAGGTTCCTAGAGGGTTGG |  |  |  |  |

Table S2. Summary table of *Ambystoma maculatum* microsatellites. AR is the rarefied allelic richness.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Locus** | **Alleles** | **AR** | **HO** | **HE** | ***F*IS** |
| *Am\_56* | 6 | 1.728 | 0.433 | 0.431 | -0.004 |
| *Am\_34* | 10 | 4.493 | 0.745 | 0.798 | 0.117 |
| *Am\_62* | 3 | 1.905 | 0.491 | 0.486 | -0.010 |
| *Am\_4* | 12 | 4.759 | 0.826 | 0.808 | -0.022 |
| *Am\_9* | 13 | 3.660 | 0.733 | 0.744 | 0.015 |
| *Am\_39* | 6 | 3.051 | 0.590 | 0.691 | 0.145 |
| *Am\_21* | 7 | 2.898 | 0.672 | 0.670 | -0.003 |
| *Am\_10* | 14 | 4.081 | 0.778 | 0.773 | -0.007 |
| *Am\_29* | 5 | 1.839 | 0.436 | 0.468 | 0.067 |
| *Am\_43* | 4 | 1.762 | 0.487 | 0.442 | -0.102 |
| *Am\_33* | 5 | 1.201 | 0.163 | 0.172 | 0.050 |
| *Am\_13* | 4 | 1.240 | 0.195 | 0.198 | 0.019 |
| *Am\_30* | 6 | 2.975 | 0.682 | 0.680 | -0.003 |
| *Am\_38* | 5 | 2.090 | 0.530 | 0.534 | 0.008 |
| *Am\_3* | 17 | 6.994 | 0.880 | 0.878 | -0.003 |
| *Am\_55* | 6 | 2.393 | 0.612 | 0.595 | -0.027 |
| *Am\_7* | 9 | 4.384 | 0.781 | 0.790 | 0.012 |
| *Am\_37* | 6 | 2.249 | 0.580 | 0.568 | -0.021 |
| Average | 7.67 | 2.983 | 0.587 | 0.596 | 0.013 |

Table S3. Pairwise distances among *Ambystoma maculatum* ponds. *F*’ST is in the lower triangle and Euclidean distance (meters) is in the upper triangle.



Table S4. Summary table of *Ambystoma annulatum* microsatellites. AR is the rarefied allelic richness.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Locus** | **Alleles** | **AR** | **HO** | **HE** | **FIS** |
| *Aa\_37\** | 6 | 2.971 | 0.476 | 0.691 | 0.312 |
| *Aa\_20* | 6 | 2.362 | 0.531 | 0.598 | 0.129 |
| *Aa\_311* | 14 | 4.910 | 0.813 | 0.822 | 0.020 |
| *Aa\_40* | 11 | 5.399 | 0.835 | 0.841 | 0.015 |
| *Aa\_25* | 9 | 4.343 | 0.780 | 0.795 | 0.024 |
| *Aa\_21* | 11 | 3.987 | 0.773 | 0.773 | -0.001 |
| *Aa\_27* | 3 | 2.527 | 0.651 | 0.622 | -0.018 |
| *Aa\_28* | 7 | 2.835 | 0.668 | 0.668 | 0.035 |
| *Aa\_86* | 3 | 1.606 | 0.372 | 0.390 | 0.059 |
| *Aa\_39* | 5 | 2.788 | 0.628 | 0.663 | 0.056 |
| *Aa\_45\** | 8 | 1.503 | 0.235 | 0.349 | 0.327 |
| *Aa\_314* | 6 | 2.404 | 0.622 | 0.602 | -0.020 |
| *Aa\_31\** | 7 | 3.130 | 0.566 | 0.707 | 0.220 |
| *Aa\_85* | 6 | 3.684 | 0.766 | 0.751 | -0.002 |
| *Aa\_36* | 10 | 3.859 | 0.749 | 0.772 | 0.113 |
| *Aa\_46* | 13 | 4.878 | 0.818 | 0.820 | 0.020 |
| *Aa\_153* | 4 | 2.116 | 0.517 | 0.545 | 0.055 |
| *Aa\_4\** | 4 | 2.341 | 0.331 | 0.601 | 0.449 |
| *Aa\_258* | 4 | 2.142 | 0.526 | 0.551 | 0.064 |
| Average† | 7.47 | 3.323 | 0.670 | 0.681 | 0.037 |

\* Locus significantly deviated from HWE expectation and omitted from downstream analyses

† Calculated without loci that deviated from HWE expectation

Table S5. Pairwise distances among *Ambystoma annulatum* ponds. *F*’ST is in the lower triangle and Euclidean distance (meters) is in the upper triangle.



Table S6. Results of linear model assessing the (log10) distance between breeding ponds in 2012 and 2013 for *Ambystoma annulatum* and *A. maculatum*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
| Intercept | 4.602 | 0.1611 | 28.569 | <0.0001 |
| Year (2013) | 0.2232 | 0.2244 | 0.995 | 0.3207 |
| Species (*A. maculatum*) | 0.5805 | 0.2358 | 2.462 | 0.0143 |
| Year\*Species | -0.7981 | 0.3117 | -2.56 | 0.0109 |

Table S7. Parameter estimates and 95% confidence intervals of mixed effects models assessing isolation-by-distance.

|  |  |  |  |
| --- | --- | --- | --- |
| *A. annulatum* |  |  |  |
|  | **β** | **Lower C.I.** | **Upper C.I.** | **t-value** |
| Intercept | 0.060 | 0.039 | 0.081 | 4.300 |
| Distance (km) | 0.022 | 0.018 | 0.027 | 10.390 |
|  |  |  |  |  |
| *A. maculatum* |  |  |  |
|  | **β** | **Lower C.I.** | **Upper C.I.** | **t-value** |
| Intercept | 0.026 | 0.012 | 0.038 | 2.701 |
| Distance (km) | 0.005 | 0.003 | 0.007 | 8.068 |

Table S8.Results of linear models fit to determine whether pond characteristics alone, isolation alone, or both pond characteristics and isolation best explained genetic differentiation. For both species, isolation was the best supported model based on AICc (see Table 4, main text).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | *A. annulatum* |  |  |  |
| Pond |  | **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
|  | Intercept | 0.06023 | 0.05722 | 1.053 | 0.315 |
|  | Predator richness | 9.472 | 7.654 | 1.237 | 0.242 |
|  | Ponds within 300m | 1.861 | 3.256 | 0.571 | 0.579 |
|  | Percent forest 300m | 0.297 | 0.695 | 0.427 | 0.678 |
|  |  |  |  |  |  |  |
| Isolation |  | **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
|  | Intercept | 0.141 | 0.010 | 13.443 | <0.001 |
|  | Isolation | -0.051 | 0.017 | -3.059 | 0.009 |
|  |  |  |  |  |  |  |
| Both |  | **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
|  | Intercept | 0.124 | 0.052 | 2.392 | 0.038 |
|  | Isolation | -0.058 | 0.022 | -2.653 | 0.024 |
|  | Predator richness | 3.676 | 6.527 | 0.563 | 0.586 |
|  | Ponds within 300m | -1.165 | 2.854 | -0.408 | 0.692 |
|  | Percent forest 300m | 0.227 | 0.559 | 0.406 | 0.693 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  | *A. maculatum* |  |  |  |
| Pond  |  | **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
|  | Intercept | 0.028 | 0.021 | 1.349 | 0.194 |
|  | Percent forest 300m | -0.075 | 0.555 | -0.136 | 0.893 |
|  | Percent canopy | 0.211 | 0.350 | 0.605 | 0.553 |
|  |  |  |  |  |  |  |
| Isolation |  | **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
|  | Intercept | 0.054 | 0.005 | 10.452 | <0.001 |
|  | Isolation | -0.039 | 0.008 | -4.884 | <0.001 |
|  |  |  |  |  |  |  |
| Both |  | **Parameter** | **β** | **S.E.** | **t-value** | **P-value** |
|  | Intercept | 0.079 | 0.017 | 4.712 | <0.001 |
|  | Isolation | -0.043 | 0.008 | -5.111 | <0.001 |
|  | Percent forest 300m | -0.575 | 0.372 | -1.545 | 0.141 |
|  | Percent canopy | 0.082 | 0.227 | 0.362 | 0.722 |