Supplementary Material.

Table S1. *Amaranthus palmeri* source populations from Georgia (GA), North Carolina (NC) and Illinois (IL). The locality of each population is indicated, as well as the population latitude (°N) and longitude (°W).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Population | Location | State | Latitude (°N) | Longitude (°W) |
| 1 | CO-GA | Colquitt Co. | GA | 31.141031 | -83.723125 |
| 2 | WO-GA | Worth Co. | GA | 31.3032 | -83.3932 |
| 3 | DO-GA | Dooly Co. | GA | 32.106095 | -83.777492 |
| 4 | MA-GA | Macon Co. | GA | 34.423982 | -84.129029 |
| 5 | LB-NC | Lumber Bridge | NC | 34.897865 | -79.047614 |
| 6 | ON-NC | Onslow Co. | NC | 34.90157 | -77.607704 |
| 7 | WN-NC | Wayne Co. | NC | 35.310539 | -78.078181 |
| 8 | RA-NC | Randolph Co. | NC | 35.55501 | -80.013807 |
| 9 | WL-NC | Wilson Co. | NC | 35.60278 | -78.071049 |
| 10 | CL-NC | Clayton | NC | 35.674307 | -78.512462 |
| 11 | JO-NC | Johnston Co. | NC | 35.674307 | -78.512462 |
| 12 | LI-NC | Linwood | NC | 35.726837 | -80.313716 |
| 13 | WA-NC | Wake Co. | NC | 35.781702 | -78.352149 |
| 14 | TA-NC | Tarboro | NC | 35.887358 | -77.443634 |
| 15 | UC-NC | UCPRS | NC | 35.897903 | -77.672789 |
| 16 | MR-NC | Martin Co. | NC | 35.979622 | -78.362871 |
| 17 | PH-NC | Pine Hill | NC | 36.360356 | -80.57341 |
| 18 | GL-IL | Gallatin Co. | IL | 37.629676 | -88.166345 |
| 19 | MD-IL | Madison Co. | IL | 38.691638 | -90.021769 |
| 20 | EF-IL | Effingham Co. | IL | 39.228864 | -88.525873 |
| 21 | CH-IL | Champaign Co. | IL | 40.074505 | -88.246533 |
| 22 | HE-IL | Henry Co. | IL | 41.447092 | -90.141592 |

Table S2. Individual plant sex assignment for *Amaranthus palmeri* in female isolation trial (ii) based on genetic markers for juveniles, and then phenotypic sex confirmation based on inflorescence observations.

|  |  |  |
| --- | --- | --- |
| Plant ID | Sex assignment  based on markers | Confirmed sex based on inflorescence |
| EFIL28\_11 | Female | Female |
| COGA27\_15 | Female | Female |
| WANC13\_2 | Female | Female |
| EFIL28\_4 | Female | Female |
| EFIL28\_15 | Female | Female |
| WANC13\_5 | Female | Female |
| ONNC5\_1 | Female | Female |
| MAGA5\_6 | Female | Female |
| ONNC5\_6 | Female | Female |
| ONNC5\_14 | Female | Female |
| MAGA5\_11 | Female | Female |
| TANC33\_6 | Female | Female |
| ONNC5\_8 | Female | Female |
| TANC33\_10 | Female | Female |
| MAGA5\_15 | Female | Female |
| EFIL28\_9 | Female | Female |
| COGA27\_9 | Female | Female |
| RANC11\_5 | Female | Female |
| EFIL28\_6 | Female | Female |
| WANC13\_14 | Female | Female |
| CHIL4\_3 | Female | Female |
| HEIL17\_13 | Female | Female |
| COGA27\_8 | Female | Female |
| EFIL28\_13 | Female | Female |
| EFIL28\_2 | Female | Female |
| COGA27\_14 | Female | Female |
| ONNC5\_13 | Female | Female |
| EFIL28\_7 | Female | Female |
| HEIL17\_5 | Female | Female |
| ONNC5\_11 | Female | Female |
| ONNC5\_12 | Female | Female |
| TANC33\_13 | Female | Female |
| MAGA5\_3 | Female | Female |
| WANC13\_4 | Female | Female |
| HEIL17\_6 | Female | Female |
| MAGA5\_7 | Female | Female |
| TANC33\_7 | Female | Female |
| WANC13\_9 | Female | Female |
| CHIL4\_1 | Female | Female |
| CHIL13\_1 | Female | Female |
| ONNC5\_10 | Female | Female |
| TANC33\_4 | Female | Female |
| RANC11\_7 | Female | Male |
| WANC13\_1 | Female | Male |
| WANC13\_3 | Female | Male |
| RANC11\_4 | Female | Male |

Table S3. Seed production (all zero) for *Amaranthus palmeri* in female isolation trial (ii), along with the number of inflorescences produced, total length of inflorescences, and average length of inflorescences.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Plant ID | Number of seeds produced | Number of inflorescences | Total length of inflorescence (cm) | Average length of inflorescence (cm) |
| EFIL28\_11 | 0 | 13 | 1362 | 104.77 |
| COGA27\_15 | 0 | 6 | 328 | 54.67 |
| WANC13\_2 | 0 | 10 | 247 | 24.70 |
| EFIL28\_4 | 0 | 15 | 1030 | 68.67 |
| EFIL28\_15 | 0 | 15 | 874 | 58.27 |
| WANC13\_5 | 0 | 5 | 346 | 69.20 |
| ONNC5\_1 | 0 | 19 | 1018 | 53.58 |
| MAGA5\_6 | 0 | 4 | 71 | 17.75 |
| ONNC5\_6 | 0 | 20 | 1267 | 63.35 |
| ONNC5\_14 | 0 | 14 | 538 | 38.43 |
| MAGA5\_11 | 0 | 17 | 1017 | 59.82 |
| TANC33\_6 | 0 | 24 | 1167 | 48.63 |
| ONNC5\_8 | 0 | 17 | 1653 | 97.24 |
| TANC33\_10 | 0 | 17 | 1273 | 74.88 |
| MAGA5\_15 | 0 | 17 | 883 | 51.94 |
| EFIL28\_9 | 0 | 25 | 1670 | 66.80 |
| COGA27\_9 | 0 | 7 | 165 | 23.57 |
| RANC11\_5 | 0 | 23 | 863 | 37.52 |
| EFIL28\_6 | 0 | 19 | 1216 | 64.00 |
| WANC13\_14 | 0 | 25 | 1639 | 65.56 |
| CHIL4\_3 | 0 | 18 | 2557 | 142.06 |
| HEIL17\_13 | 0 | 16 | 357 | 22.31 |
| COGA27\_8 | 0 | 0 | 0 | 0.00 |
| EFIL28\_13 | 0 | 25 | 1200 | 48.00 |
| EFIL28\_2 | 0 | 10 | 242 | 24.20 |
| COGA27\_14 | 0 | 11 | 648 | 58.91 |
| ONNC5\_13 | 0 | 22 | 791 | 35.95 |
| EFIL28\_7 | 0 | 16 | 658 | 41.13 |
| HEIL17\_5 | 0 | 20 | 1167 | 58.35 |
| ONNC5\_11 | 0 | 18 | 521 | 28.94 |
| ONNC5\_12 | 0 | 9 | 230 | 25.56 |
| TANC33\_13 | 0 | 22 | 2176 | 98.91 |
| MAGA5\_3 | 0 | 17 | 521 | 30.65 |
| WANC13\_4 | 0 | 1 | 37 | 37.00 |
| HEIL17\_6 | 0 | 32 | 1812 | 56.63 |
| MAGA5\_7 | 0 | 7 | 453 | 64.71 |
| TANC33\_7 | 0 | 16 | 244 | 15.25 |
| WANC13\_9 | 0 | 30 | 808 | 26.93 |
| CHIL4\_1 | 0 | 33 | 1137 | 34.45 |
| CHIL13\_1 | 0 | 33 | 1271 | 38.52 |
| ONNC5\_10 | 0 | 24 | 573 | 23.88 |
| TANC33\_4 | 0 | 30 | 444 | 14.80 |

Table S4. Best-fit models for variation in each of the seven morphological features of *Amaranthus palmeri* measured in female and male plants from 19 populations from eastern North America. Latitude, and population x sex were not significant in all cases; thus, models were reduced to a single fixed effect ‘Sex\_Bagging’ (variable that combines whether a plant is female or male, and whether females were bagged) and the random effect population. Planned contrasts were performed between each level of the ‘Sex\_Bagging’ variable to test whether there is a significant difference between females that are unbagged vs. bagged (F-Fbag), unbagged females and unbagged males (F-M), and bagged females and unbagged males (Fbag-M).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  | Contrasts | | |
|  | Variable | LMM model *F* | *df* | Model *P* | Pop’n *P* | F-Fbag *P* | F-M *P* | Fbag-M *P* |
| (A) | Height | 6.5302 | 2, 528.67 | 0.0016 | 0.0076 | 0.9943 | 0.0166 | 0.0151 |
| (B) | Stem diameter | 0.4374 | 2, 36.89 | 0.649 | 0.3445 | 0.6339 | 0.7538 | 0.9609 |
| (C) | Total 2° branch length | 17.825 | 2, 78.27 | 4.178e-07 | 0.3042 | <0.0001 | 0.0271 | <0.0001 |
| (D) | Total inflorescence length | 17.218 | 2, 65.19 | 9.913e-07 | 0.2821 | 0.0001 | 0.9643 | <0.0001 |
| (E) | No. inflorescences | 2.0569 | 2, 50.10 | 0.1385 | 0.9885 | 0.9997 | 0.2278 | 0.1675 |
| (F) | No. axillary inflorescences | 77.625 | 2, 60.27 | < 2.2e-16 | 0.3494 | 0.3909 | <0.0001 | <0.0001 |
|  |  | GLM |  |  |  |  |  |  |
| (G) | 2° branches (binary) | χ2= 62.381 | 2 | 2.846e-14 |  | <0.0001 | 0.2659 | <0.0001 |

A graph showing the number of dna content

Description automatically generatedA diagram of a dna content

Description automatically generated

Figure S1. Linear relations between mean DNA content (pg) as estimated in this study and mean EPSPS copy number (estimated in Yakimowski et al. 2023) (a) across populations of *Amaranthus palmeri* from Georgia, North Carolina and Illinois and (b) estimated separately for population (GA – Georgia=red; NC – North Carolina=blue; IL – Illinois=green).

A close-up of a dna test

Description automatically generated

Figure S2. Example of male-specific genetic marker PCR product. Using the 100bp ladder on the far-left column of both rows, a band present of size ~100bp was used to assign sex phenotype as male (noted with a dashed line above). Individuals associated with this fragment were assumed male and discarded.