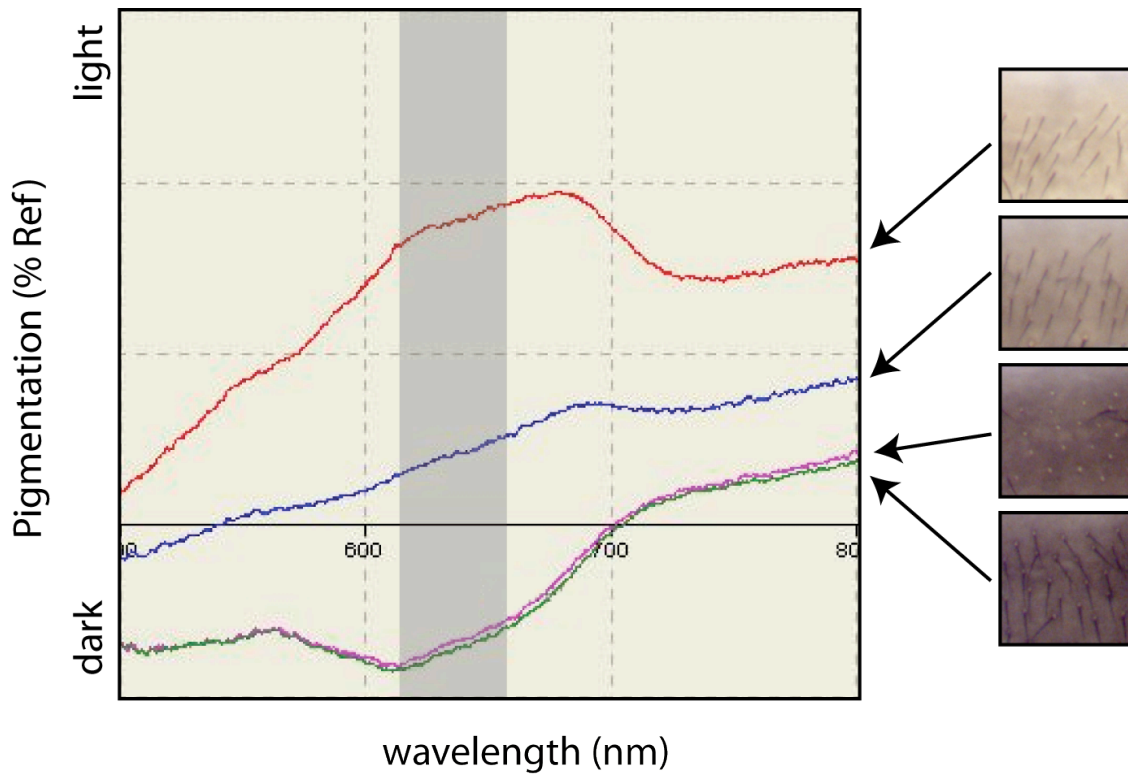
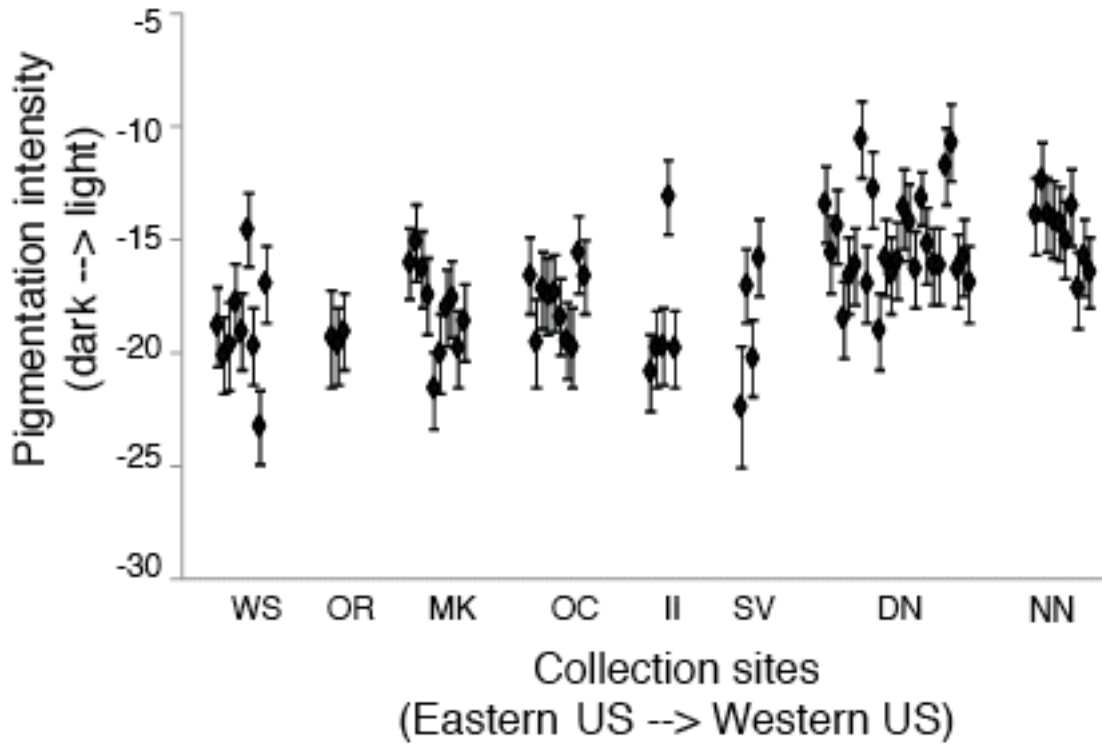


Supplementary Figure 1. Image-based quantification of pigmentation intensity.

Greyscale images of abdominal cuticles (segments A3, A4, and A5) from five adult flies are shown. Lines show the average pixel intensity at each horizontal position within each segment. Note the increased intensity values along the dorsal midline of most cuticles where pigmentation is lighter. Pigment quantification for Dataset A was limited to the regions flanking this lighter midline region (arrows).



Supplementary Figure 2. Pigmentation quantification using a custom-built fiber-optic probe for spectrometry. Regions from four reference cuticles used to calibrate spectrophotometer each day are shown. The percentage of light reflected at each wavelength is shown. As described in Materials and Methods, the reflectance of light between 610 and 660 nm (gray) was used as a measure of pigmentation for Dataset B.



Supplementary Figure 3. Line-specific least-squares means (and 95% confidence intervals) for pigmentation in Dataset B. Collection sites are arranged by increasing degrees west longitude.

Supplemental Table 1. Origins of *Drosophila americana* isofemale lines. Lines with no citation are previously unpublished.

| Collection Site | Lines | Year ¹ | Lat ² | Long ³ | Alt ⁴ | Temp ⁵ | RH ⁶ |
|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|------------------|-------------------|------------------|-------------------|-----------------|
| Anderson, IN | A00 ^{6*} | unknown | 40.1 | 85.6 | 279 | 11.1 | 67.8% |
| Poplar, MT | A01 ^{6*} | 1947 | 48.1 | 105.2 | 593 | 4.7 | 63.9% |
| Lake Champlain, VT | A04 ^{6*} | 1948 | 44.6 | 73.4 | 28 | 6.1 | 68.0% |
| Myrtle Beach, SC | A09 ^{6*} | 1961 | 33.7 | 78.9 | 6 | 19.6 | 69.2% |
| Augusta, AR | AA99.6 ^{7*} | 1999 | 35.3 | 91.4 | 59 | 15.6 | 68.6% |
| Duncan, NE | DN01.12 ^{8*} , DN00.2 ^{8*} , DN00.4 ^{8*} , DN07.4, DN07.6, DN07.14, DN07.16, DN07.18, DN07.20, DN07.24, DN07.34, DN07.42, DN07.08x05, DN07.10x07, DN07.12x09, DN07.26x19, DN07.28x21, DN07.32x25, DN07.40x31, DN07.44x33, DN07.48x37, DN07.52x41, DN07.54x43, DN07.56x45, DN07.60x47, DN07.62x49, DN07.64x53, DN07.66x55 | 2000 (DN00.X) 2001 (DN01.X) 2007 (DN07.X) | 41.4 | 97.5 | 469 | 8.9 | 67.9% |
| Floodgate Park, AR | FP99.32 ^{7*} | 1999 | 34.2 | 91.1 | 43 | 16.6 | 69.1% |
| LaSalle Fish & Wildlife Area, IN | II07.2, II07.4, II07.6, II07.8, II07.10 | 2007 | 41.2 | 87.5 | 192 | 10.3 | 68.5% |
| Hawkeye Wildlife Area, IA | IR04.10 ^{9*} | 2004 | 41.8 | 91.7 | 208 | 10.2 | 65.7% |
| Lake Ashbaugh, AR | LA99.48 ^{7*} | 1999 | 36.3 | 90.8 | 79 | 14.8 | 68.0% |

| | | | | | | | |
|--------------------------------------|------------------------------------------------------------------------------------------|------------------------------------|------|------|-----|------|-------|
| Muscatatuck Natl Wildlife Refuge, IN | MK07.16, MK07.18, MK07.20, MK07.22, MK07.24, MK07.30, MK07.36, MK07.38, MK07.42, MK07.46 | 2007 | 38.9 | 85.8 | 166 | 13 | 67.7% |
| Niobrara, NE | NN07.6, NN07.8, NN07.10, NN07.14, NN07.16, NN07.17, NN07.18, NN07.22, NN07.26, NN07.28 | 2007 | 42.7 | 98.1 | 448 | 8.1 | 67.7% |
| Patoka Natl Wildlife Refuge, IN | OC07.2, OC07.12, OC07.14, OC07.16, OC07.18, OC07.24, OC07.26, OC07.28, OC07.32, OC07.34 | 2007 | 38.3 | 87.3 | 136 | 13.4 | 67.3% |
| Ottawa Natl Wildlife Refuge, OH | OR01.52 ^{10*} , OR07.6, OR07.10, OR07.12 | 2001 (OR01.X) 2007 (OR07.X) | 41.6 | 83.2 | 175 | 10.2 | 68.4% |
| Saulsbury Bridge Rec. Area, IA | SB02.6 ^{9*} | 2002 | 41.5 | 91.2 | 186 | 10.2 | 65.7% |
| Swan Lake Natl Wildlife Refuge, MO | SV07.10, SV07.16, SV07.18, SV07.26 | 2007 | 39.6 | 93.2 | 202 | 11.8 | 66.8% |
| Killbuck Marsh, OH | WS07.2, WS07.6, WS07.8, WS07.10, WS07.12, WS07.14, WS07.16, WS07.18, WS07.20 | 2007 | 40.7 | 82.0 | 265 | 10.3 | 70.1% |

Lines included in Dataset A are indicated with an asterisk. All other lines are included in Dataset B.

¹ Year female used to initiate isofemale line was collected from the wild.

² Latitude of collection site (°N)

³ Longitude of collection site (°W)

⁴ Altitude of collection site (meters)

⁵ Mean annual daily temperature (from NASA Surface Meteorology and Solar Energy)

⁶ Mean annual relative humidity (from NASA Surface Meteorology and Solar Energy)

⁷ Drosophila Species Stock Center, San Diego, CA (formerly Tucson, AZ)

⁸ McAllister, 2002

⁹ McAllister, 2003

¹⁰ McAllister and Evans, 2006

¹¹ McAllister, 2001