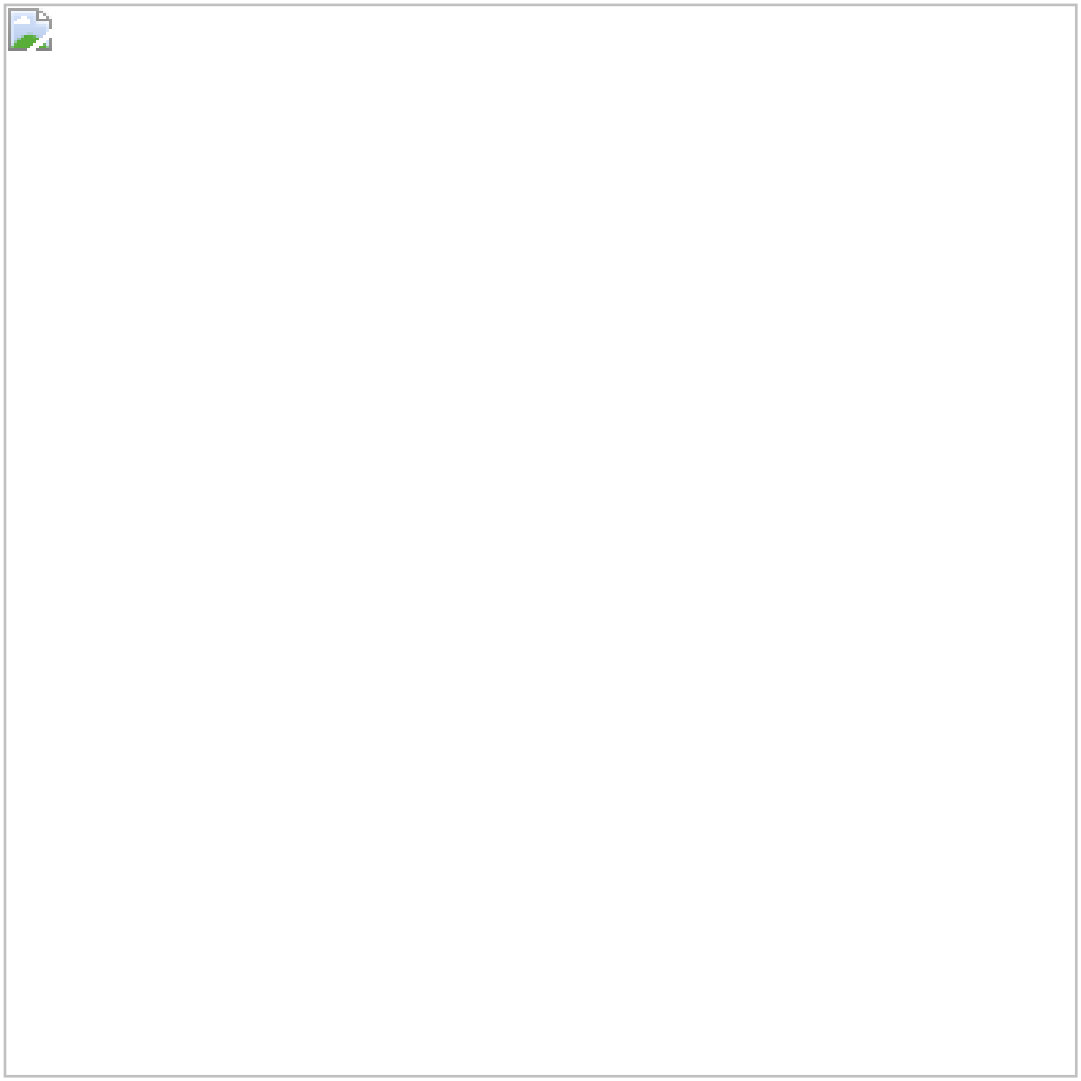

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Density and temperature-dependent melanization of fifth-instar *Melanoplus sanguinipes*: interpopulation comparisons

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Abstract

Behavioral thermoregulation, by basking in sunlight, is very common in acridids. The resulting increased body temperatures may be beneficial by accelerating feeding and developmental rates and by enhancing disease resistance. The absorption of solar energy is affected by the degree of cuticular melanization. In this paper, we quantify differences in color and thermoregulation between subarctic and temperate populations of *Melanoplus sanguinipes*. We also quantify effects of rearing temperatures and density on melanization of 5th instars. Alaskan grasshoppers tended to be darker than those from Idaho when reared under the same conditions and Alaskan grasshoppers also responded to cooler rearing temperatures by increased pigmentation. Density-dependent melanization was evident in both populations. Increased pigmentation enhanced the ability to thermoregulate. Absorption of wavelengths in the near infrared region was similar to that in the visible range, confirming that visible color is a reliable measure of relative absorption of total solar irradiance. Strong selection for efficient thermoregulation at high latitudes was suggested by the population differences in pigmentation.

Key words

thermal ecology, thermoregulation, subarctic, Orthoptera, Acrididae

Introduction

The ability to elevate body temperature above ambient is crucial for many insects in temperate climates (Heinrich 1993). Probably the most common method of thermoregulation is basking in sunlight, which is a very common behavior in grasshoppers (Kemp 1986, Chappell & Whitman 1990, Willott 1997, Lactin & Johnson 1996). Many benefits acrue from elevated body temperatures. Feeding, growth, and fecundity may be enhanced (Lactin & Johnson 1995, Harrison & Fewell 1995, Ashby 1998, Ellers & Boggs 2004). Some species of insects may extend their range and persist in climates where they otherwise could not without thermoregulating (Whitman 1988, Bryant *et al.* 2002, Ellers & Boggs 2004, Fielding 2004). Grasshoppers infected by various pathogens frequently thermoregulate to higher than normally preferred body temperatures (behavioral fever), and in doing so, are more successful in surviving the infection (Carruthers *et al.* 1992; Inglis *et al.* 1996, 1999; Elliot *et al.* 2002; Ouedraogo *et al.* 2004).

Body color influences radiative absorption (Digby 1955, Watt 1968, Chappell & Whitman 1990, Forsman 2000). Melanization in insects may be affected by genetics (Kingsolver & Huey 1998, Forsman *et al.* 2002), immune reactions (Cotter *et al.* 2004, Barnes & Siva-Jothy 2000), and environmental conditions, including population density (Applebaum & Heifetz 1999, Hagen *et al.* 2003), and rearing temperature (Parker 1930, Solensky & Larkin 2003). Parker (1930) and Brett (1947) noted that nymphs of *Melanoplus sanguinipes* F. were darker when reared at lower temperatures. Brett (1947) also noted darker colors in *M. sanguinipes* when raised in high density populations. We have observed similar effects in laboratory colonies of *M. sanguinipes*, derived from populations in Idaho and Alaska. *M. sanguinipes* inhabits a very wide range of habitats (Hebard 1929, Banfill & Brusven 1973), making it a good subject for comparative studies of adaptations to different climates. The objectives of this study were to quantify interpopulation differences in coloration and the effects of population density and temperature regime in 5th instar nymphs of *M. sanguinipes*, and to relate pigmentation to thermoregulatory efficiency.

Methods

Laboratory colonies were initiated with >200 individuals, collected near Lewiston, Idaho and Delta Junction, Alaska, as 4th and 5th instars, in mid-July 2003. Grasshoppers were collected about 5 km southwest of the National Oceanic and Atmospheric Administration (U.S. Department of Commerce) weather station at Lewiston, Idaho (lat 46.38° N, long 117.02° W, 450 m elevation) and about 20 km SE of the weather station at Delta Junction, Alaska (lat 64.00° N, long 145.73° W, 400 m elevation) (WRCC, 2004). All experiments were conducted on F_1 and F_2 generation offspring.

To examine the effects of rearing temperature on pigmentation, grasshoppers were raised at 33 (±1)°C constant temperature and at diurnally alternating temperatures of 33°C (±1) for 12 h and 15 (±1)°C for 12 h. Grasshoppers were kept in cylindrical tubes (10.8 cm diameter × 46 cm length) of acetate with the ends covered with aluminum insect screening, and fed wheat bran and romaine lettuce *ad libitum*. For each treatment, 10 grasshoppers from at least 5 different sets of parents were reared in each of 3 tubes.

In experiments to determine effects of crowding on pigmentation, grasshoppers were reared at the 33/15°C alternating temperatures in tubes as described above, except that 3 tubes for each population contained 50 nymphs, 3 tubes contained 20 nymphs, and 6 tubes contained 5 nymphs each.

Spectral Analysis.—Three nymphs, representative of a range of melanization, were selected for spectral analysis. Reflectance was measured at wavelengths from 350 to 2500 nm with a spectroradiometer (FieldSpec Pro JR, Analytical Spectral Devices, Inc., Boulder, Colorado, USA) having spectral resolution of 3 nm for wavelengths from 350 to 1000 nm and 10 nm from 1000 to 2500 nm. The optical

setup was as recommended by the instrument manufacturers (ASD, 1997): samples were illuminated with a tungsten quartz halogen filament lamp (Lowel pro-lamp; 50W bulb; ~3200 K color temperature); the sample grasshopper was horizontal; the light beam was oriented 30° from vertical, at a distance of about 30 cm from the sample. Reflected light was collected with a 25° field-of-view fiber optic probe angled at 30° from vertical and perpendicular to the axis of illumination at a distance of 5 mm from the sample. Each spectrum obtained from the grasshoppers was a mean of 20 scans. Because the grasshoppers were not uniformly pigmented, 5 spectra were obtained from different views of each grasshopper: 2 lateral views centered on the thorax, 2 lateral views centered on the abdomen, and 1 dorsal view centered on the thorax and wing pads. The 5 spectra were averaged to obtain an overall reflectance spectrum for each grasshopper. Before reading each sample, 10 white reference spectra were recorded using a calibrated spectralon reference surface (Labsphere®, Sutton, NH), placed at the same distance from the foreoptic as the grasshoppers. Reflectance readings for each wavelength band were expressed as a proportion of the white reference readings. Radiation absorbed was calculated as 1-reflectance. The relative amount of solar energy (W m^{-2}) absorbed by each nymph was estimated as the sum of the products of absorption and irradiation at 1 nm bandwidths. Irradiance was taken from the standard reference spectrum for direct plus diffuse solar radiation at 1.5 atmospheres, incident on a sun-facing, 37° tilted surface (ASTM, 2003).

Color measurements.— Melanization was quantified by analysis of scanned images of 5 to 10 fifth-instar nymphs chosen haphazardly from each tube, except very slow-developing individuals were excluded, for a total of 15 to 25 nymphs for each treatment. Three- to 6-d old 5th instar nymphs were killed by freezing, and scanned after warming to room temperature and blotting condensation. Images, 16-bit grayscale in TIF format, of these nymphs were taken with a scanner (Expression 1600, Epson America, Inc., Long Beach, California, USA), set to a resolution of 23.6 dots per mm (600 dots per inch). The scanner incorporated 2 light sources (Regent Instruments, Inc., Quebec, QC., Canada), one under the cover of the scanner (above the grasshoppers) and the other below as part of the scanner main body, to eliminate shadows that could confuse the analysis. Because typical basking posture of acridid nymphs is perpendicular to the sun's rays with the hind leg lowered to expose the abdomen to the light, the nymphs were positioned laterally (when viewed from below) on the scanner bed with their hind legs removed. The images were analysed with Assess: Image Analysis Software for Plant Disease Quantification (American Phytopathological Society, St. Paul, Minnesota, USA), by generating a histogram of pixel values for the full lateral view of each individual. Pixel values ranged from 0 (black) to 255 (white). Mean pixel value was derived from these histograms. Although the histograms of some individuals showed skewed or bimodal distributions, preliminary analyses showed that similar results were obtained using mean pixel value, as with the median or percentage of pixels below a value of 100.

Temperature Gain.— A class B solar simulator with a 150 W xenon lamp (model SS50B, Photo Emission Tech., Inc., Newbury Park, California, USA) was used to measure heat gain in the same dead grasshopper nymphs immediately after being scanned. Xenon lamps produce a spectrum that approximates the solar spectrum. At a distance of 26 cm, the solar simulator provided radiation of about 900 W m^{-2}, as measured with a pyranometer (LI-200SA, LiCor Inc., Lincoln, Nebraska, USA). A copper-constantin thermocouple was inserted in the abdomen of the grasshopper until the tip of the probe was near the center of the thorax. Grasshoppers were oriented perpendicular to the light beam. After 6 minutes, body temperature had reached equilibrium and was recorded. A separate thermocouple, just outside the area of illumination, simultaneously recorded ambient temperatures.

Data Analysis.— Mean pixel values and body temperature gain were found to be normally distributed and so no data transformations are involved. Analysis of variance was conducted with mean pixel value and temperature gain as dependent variables, and population origin and either temperature regime or rearing density as independent variables. Linear regression related temperature gain to mean pixel value. All statistical analyses were made using the GLM procedure of SAS-STAT (SAS Institute, 2001). Results are reported in terms of least-squares means and standard errors because of uneven sample sizes.

Results

Absorption spectra from representative nymphs show that absorption in the near-infrared (from 800 to 1300 nm) varies among individuals in the same manner as in the visible range (from 350 to 800 nm) (Fig. 1), indicating that color measurement in the visible range is a reliable indicator of the relative ability of grasshopper nymphs to thermoregulate. All nymphs absorbed radiation above 1300 nm to much the same degree, but irradiance at wavelengths from 1300 to 2500 nm accounts for only about 12% of the total energy of the standard reference spectrum described above (ASTM, 2003). Values for the proportion of total solar irradiance absorbed were similar to that estimated by Lactin & Johnson (1997): e.g., 0.722 compared to, for example, 0.71 by individual #2 in Fig. 1.

Melanization (mean pixel value) was related to gain in body temperature (r^2=0.38, n=196, F=121, p<0.0001) (Fig. 2). The variation in temperature gain that could not be accounted for by melanization may result from variation in mass among grasshoppers, slight variation in the location of the temperature probe, evaporative cooling from the probe insertion point, or a skewed distribution of pixel values.

Population source had a highly significant effect on mean pixel value and temperature gain (Tables 1, 2) in both experiments. Alaskan grasshoppers were darker and had greater temperature gain than the Idaho population in all treatments (Figs 3, 4).

The temperature regimes at which nymphs were reared had a significant effect on mean pixel value and on temperature gain (Table 1), with grasshoppers tending to be darker when experiencing cool temperatures during the diurnal cycle. There was a weak interaction effect between population and rearing temperature. Alaskan grasshoppers showed significant differences in mean pixel value and gain in body temperature due to rearing temperature, whereas the differences were not significant in the Idaho population (Table 1, Fig. 3).

Density at which the grasshoppers were reared had a significant effect on mean pixel value and temperature gain (Table 2), with grasshoppers tending to be darker and showing greater temperature gain when reared under higher densities (Fig. 4a, b). The interaction effect between population and density was not significant (Table 2).

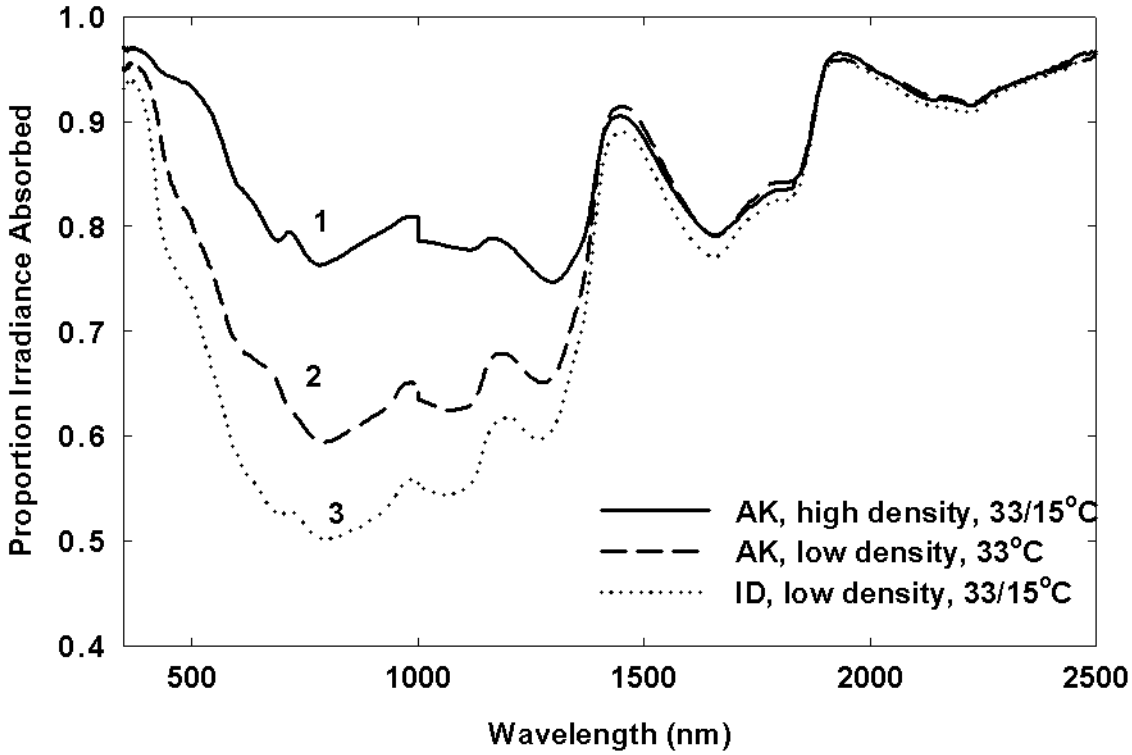

Fig. 1. Absorption spectra from 3 representative 5th instar *M. sanguinipes*. For individuals 1, 2, and 3, respectively, mean pixel values were 103, 125, and 148; temperature gain was 12.1, 9.8, and 8.7°C; percentage of total solar irradiance absorbed was 84, 71, and 63%. Percentage aborption of solar irradiance was 87, 74, and 66% in the visible wavelengths (350 to 800 nm), and 78, 63, and 51% in the NIR (800-1300nm), for individuals 1, 2, and 3, respectively.

Discussion

Population origin, rearing temperature, and density influenced pigmentation of 5th instar *M. sanguinipes*. Alaskan individuals tended to be darker than those from Idaho. Subartic environments have short summers, which makes rapid development imperative. This presumably selects for efficient thermoregulation. Even though at high latitudes the sun is above the horizon for 20 or more hours per day during the summer, solar irradiance is never as intense as at lower latitudes, increasing selection pressure for efficient thermoregulation.

Density and temperature-dependent pigmentation have been noted before for *M. sanguinipes* (Parker 1930, Brett 1947), but never quantified. In other species of acridids, mainly locusts, density dependent color changes have been associated with polymorphic phase change (Pener 1991, Sword *et al.* 2000). In contrast, changes in pigmentation in *M. sanguinipes* appear to be graded. Increased melanization in other species of insects, mainly Lepidoptera and Coleoptera, has been associated with immune responses (Wilson *et al.* 2001, Cotter *et al.*, 2004), possibly because encapsulation of pathogens involves some of the same metabolic pathways (Wilson *et al.* 2001). Disease epidemics are much more likely in high density populations, and so population density may serve as a signal to invest more resources towards disease resistance before pathogens appear (Wilson & Cotter 2006). Thus, cuticular melanization may be a side-effect of a density-dependent immune response. Alternatively,

Table 1. ANOVA results for effects of population origin and rearing temperature on melanization (mean pixel value) and gain in body temperature in 5th instar nymphs of *Melanoplus sanguinipes*.

Factor	Mean Square	F-value	p
Melanization (d.f. = 1,68)			
Population	11560	92.21	<0.0001
Temperature	1196	9.54	0.0029
interaction	388	301	0.0829
Temperature Gain (d.f. = 1, 62)			
Population	33.9	39.89	<0.0001
Temperature	4.8	5.62	0.0209
Interaction	2.9	3.39	0.0703

Table 2. Results of ANOVA for effects of population origin and crowding on melanization (mean pixel value) and gain in body temperature in 5th instar nymphs of *Melanoplus sanguinipes*.

Factor	Mean Square	F-value	p
Melanization			
Population, d.f. = 1, 114	26 557	196.7	<0.0001
Density, d.f. = 2, 114	1891	14.01	<0.0001
interaction, d.f. =2, 114	268	1.98	0.1424
Temperature Gain			
Population, d.f. = 1, 112	69.6	66.5	<0.0001
Density, d.f. = 2, 112	21.2	20.33	<0.0001
Interaction, d.f. = 2, 112	0.4	0.37	0.69

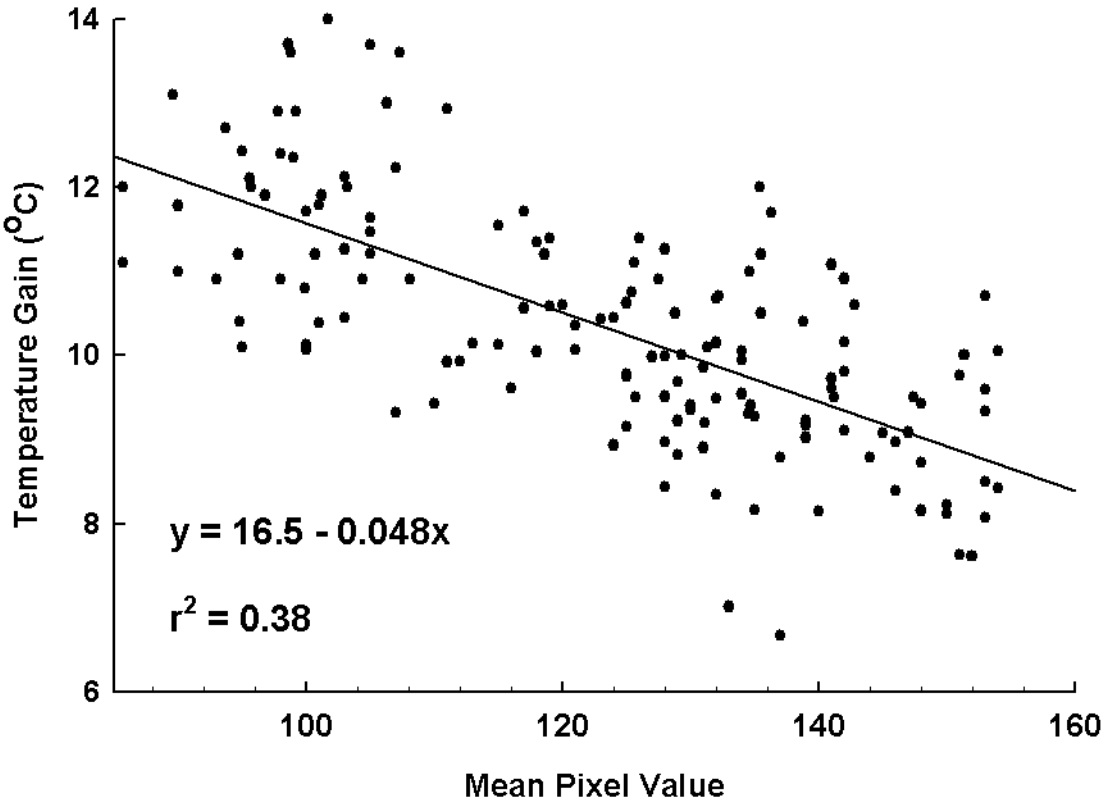

Fig. 2. Relationship between melanization and increase in body temperature when exposed to irradiance (approximately 900 W m^{-2}) from a xenon lamp with spectral qualities similar to the solar spectrum.

increased melanization may directly augment disease resistance by enhancing behavioral fever. By increasing absorption of solar energy, pigmentation in grasshoppers may allow them to attain febrile body temperatures at lower levels of solar irradiance than would otherwise be possible.

The biophysical model of Lactin & Johnson (1997) showed the importance of body size and orientation for thermoregulation. The results of these experiments show that color is also an important factor determining efficiency of thermoregulation. The interpopulation differences suggest both a genetic and an environmental basis for melanization. Phenotypic variation in melanization probably represents reaction norm evolution in response to various selection pressures, possibly including short growing seasons and pathogens.

Prediction of developmental stages in the field is important for pest management. Entomologists spend considerable effort developing phenological models for pest management programs and population models. The differences between populations in pigmentation, in addition to interpopulation differences in developmental rates (Dingle *et al.* 1990, Fielding 2004), suggest that phenological models need to be region specific.

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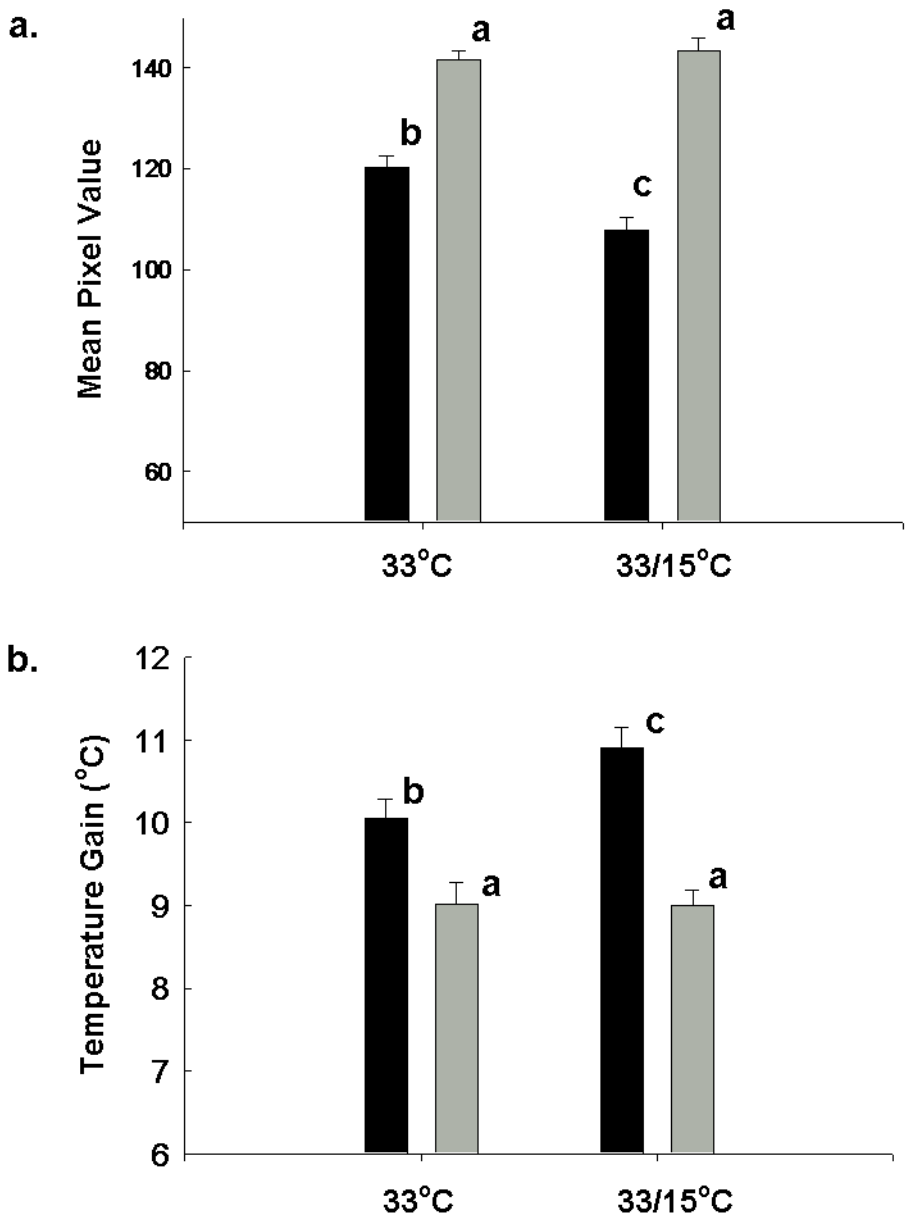

Fig. 3. Differences in color and temperature gain of 5th instar *M. sanguinipes* reared under different temperature regimes. Lower pixel values correspond to darker color. Least-squares mean (with s.e.) pixel values (a); and temperature gain (b), for Alaskan (black bars) and Idaho (gray bars) populations. Treatment means with different letters are significantly different ($p<0.01$, pairwise T-test). n= 15 to 25 for each mean.

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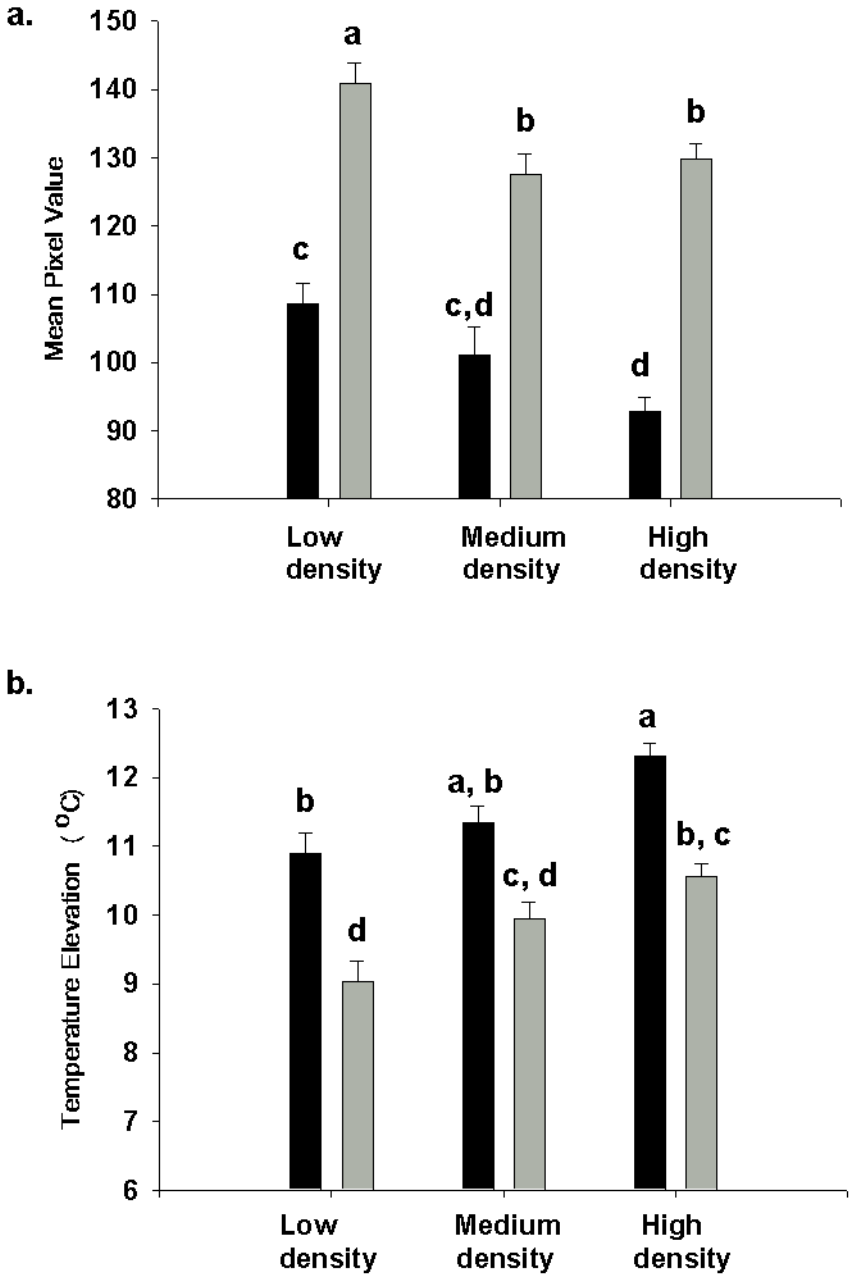

Fig. 4. Differences in color and temperature gain of 5th-instar *M. sanguinipes* reared under different population densities. Lower pixel values correspond to darker color. Least-squares mean ± s.e. pixel values (a); and temperature gain (b), for Alaskan (black bars) and Idaho (gray bars) populations. Treatment means with different letters are significantly different ($p<0.01$, pairwise t-test). n= 15 to 25 for each mean.

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