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Short Communication

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## A Method for Estimating the Age of Bees: Age-Dependent Wing Wear and Coloration in the Wool-Carder Bee *Anthidium manicatum* (Hymenoptera: Megachilidae)

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Accepted January 29, 1993; revised March 16, 1993

**KEY WORDS:** age; *Anthidium manicatum*; bees; wing wear.

The estimation of age of field-caught insects has been a persistent problem in behavioral and ecological studies of insects. For bees, Michener *et al.* (1955) and Ordway (1965) suggested the use of wing wear and mandibular wear as crude indicators of relative age. Michener (1953) and Severinghaus *et al.* (1981) proposed hair coloration as a third measure of age. For wasps, Richards (1978) and Ishay and Shimony (1982) documented gradual fading of cuticular pigment with age. While all of these measures appeared to provide valid estimates of relative age, none of these studies attempted to quantify the relationship between estimated wear or coloration and actual age. Menzel *et al.* (1969) investigated circadian, cuticular depositions to predict age in the honey bee *Apis mellifera* and reported that the number of cuticular layers corresponded closely to the actual age of honeybees during the first 15 days of a bee's life but not thereafter. Though superior in predictive power to those methods that are based on wear and coloration, this invasive method proves impractical to the behavioral ecologist interested in the study of living organisms.

We present here a noninvasive method for accurately predicting the age of field-caught bees. In particular, we propose a standard scale for the evaluation of wing wear in bees and test the reliability and validity of this method in the solitary wool-carder bee *Anthidium manicatum*.

Originally a palaearctic species, *A. manicatum* was introduced to the eastern United States during this century (Jaycox, 1967; Pechuman, 1967). Like other

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species of its genus, *A. manicatum* exhibits a resource defense mating system, with males defending territories (patches of flowers) visited by females for the collection of pollen, nectar, and nest material (Severinghaus *et al.*, 1981; Mueller, 1987; Wirtz *et al.*, 1988). Territorial males aggressively repel conspecific males and heterospecifics intruding into their territories. Nests occur away from territories and are tended by solitary females. As is expected under such a mating system, males are considerably larger in size than females.

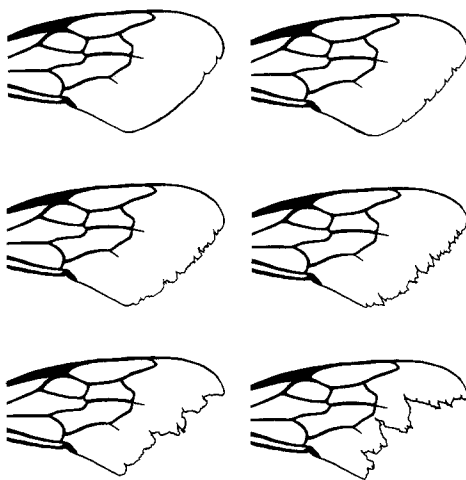
We monitored the age-dependent progression of wing wear and hair coloration in a bivoltine population of *Anthidium manicatum* (Hymenoptera: Megachilidae) over a period of 129 days between 31 May and 5 October 1988. The study population inhabited several flower gardens located on the campus of Cornell University, Ithaca, New York. Mueller (1987) presents a detailed description of the study sites.

Male and female *A. manicatum* were captured with a handheld net at foraging sites, placed individually into scintillation vials, and chilled on ice to ensure complete immobilization during subsequent handling. Wing wear, as apparent in serrations or even missing chunks of the apical and posterior margins of the anterior wing, was judged with respect to the following standards of wing wear (Fig. 1).

WW 0: Wing margin completely intact.

WW 1: Wing margin showing one or two nicks.

WW 2: Wing margin showing 3 to 10 nicks.



**Fig. 1.** Standards of wing wear used for the determination of age in *Anthidium manicatum*. See the text for the defining criteria of the various standards.

- WW 3: Wing margin almost completely serrated with more than 10 nicks, but at least some original margin intact.
- WW 4: Wing margin completely serrated with excisions less than half the width of the distal submarginal cell. No original margin intact.
- WW 5: Wing margin completely serrated with excisions more than half, but less than the entire width of the distal submarginal cell.
- WW 6: Wing margin showing major excisions greater than the width of the distal submarginal cell. Excisions may reach the distal cross-veins.

Wing margins of both anterior wings were inspected using a 10× magnifying glass and evaluated separately according to the seven standards. Scores from both wings were averaged. Only in 4.8% of the measurements did the estimates from the two wings differ by more than one standard category. Large, single excisions suggesting a single accident rather than gradual wear were ignored.

The coloration of the hair anterior to the tegula was judged on a scale from 1 to 5, ranging from brightly orange over orange, orange-brown, brown-gray, to white. To standardize the coloration scale, we found it best first to gain an impression of the range of coloration existing in *A. manicatum*, then subjectively divide this range into five subcategories.

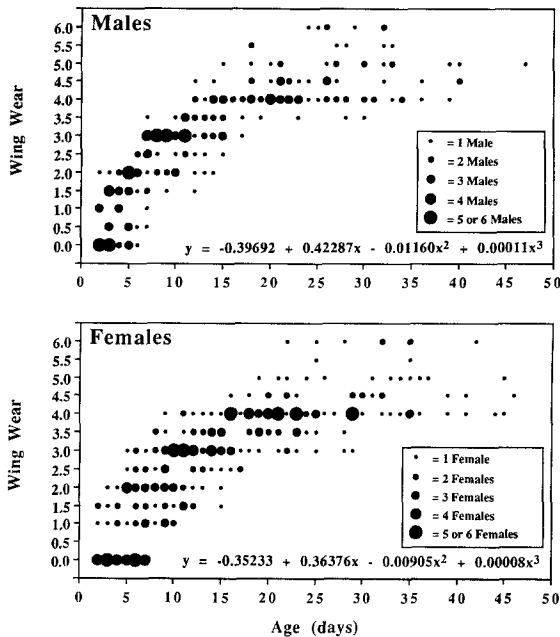
After inspection of the wings and tegular hair, we measured the head width of all bees to the nearest 0.01 mm with calipers. Head width correlates highly with dry weight in male and female *A. manicatum* [ $r_{\text{male}} = 0.94$ ,  $P < 0.001$ ;  $r_{\text{female}} = 0.88$ ,  $P < 0.001$  (Mueller, 1987)] and therefore represents a valid predictor of a bee's size. Following size determination, bees were placed into a restrainer (for a description, see Mueller *et al.*, 1992) to facilitate code-marking with individual color combinations of fast-drying Testors enamel. After the enamel had dried, bees were released at the site of capture.

*Reliability of Wing Wear and Hair Coloration Estimates.* We examined the repeatability of the aging procedure by independently evaluating a sample of 25 male and 25 female *A. manicatum* in the field. Scores of both wing wear and hair coloration derived from one investigator were then related to the scores derived from the other investigator. The correlation between wing wear estimates taken blindly by the two independent investigators was  $r = 0.91$  ( $P < 0.001$ ). The correlation for tegular hair coloration estimates was  $r = 0.84$  ( $P < 0.001$ ). The categories of wing wear and coloration therefore are sufficiently distinct to guarantee repeatability and represent reliable gradations of wear and coloration in *A. manicatum*.

*Validity of Wing Wear and Hair Coloration Estimates.* We revisited each study site at intervals from 1 to 4 days throughout the study season, captured all marked and unmarked *A. manicatum* encountered, and evaluated each bee as described above. Repeated measures were analyzed for time trends using

multiple regression techniques. We restricted the analyses to those bees (55 males and 91 females) that showed no wing wear at the time of marking (first capture). These individuals without wing wear were assumed to have emerged within the few days since last visiting a study site and therefore were of young age (not older than about 4 days). A total of 292 and 377 measurements was taken on these 55 males and 91 females, respectively. Individual bees were recaptured between 1 and 13 times each and at intervals of between 1 and 25 days. Males were recaptured more frequently (mean =  $5.3 \pm 3.18$  measurements) and at shorter intervals (mean =  $5.0 \pm 4.23$  days) than females (mean =  $4.1 \pm 2.50$  measurements; mean =  $6.9 \pm 3.83$  days).

For both the sample of 55 males and the sample of 91 females, wing wear showed a significantly positive, nonlinear relationship with age (Fig. 2). For both males and females, a cubic polynomial model provided a significantly better fit than any higher-order or lower-order model, yielding adjusted multiple  $R^2$  values of 0.869 for the male model ( $F = 477.5$ ,  $df = 3/288$ ,  $P < 0.0001$ ) and 0.845 for the female model ( $F = 682.1$ ,  $df = 3/373$ ). Likewise, tegular hair



**Fig. 2.** Progression of wing wear over time for 55 male (top) and 91 female (bottom) *A. manicatum*. Wing wear ranges from no wear (WW 0) to extreme wear (WW 6). Wing wear shows a significant nonlinear relationship with age ( $P < 0.0001$  for both sexes) but progresses significantly faster in males than in females ( $P < 0.005$ ).

coloration showed a significantly positive, nonlinear relationship with age in both sexes (Fig. 3). As in wing wear, coloration progressed as a cubic polynomial function in both females (adjusted  $R^2 = 0.722$ ,  $F = 326.1$ ,  $df = 3/373$ ,  $P < 0.0001$ ) and males (adjusted  $R^2 = 0.815$ ,  $F = 316.1$ ,  $df = 3/287$ ,  $P < 0.0001$ ). Wing wear scores below 3 and hair coloration scores below 4 provided the most accurate estimates in both males and females (Figs. 2 and 3). These scores cover the first 15–20 days of the bees' lives. Both wing wear and coloration progress rapidly during this time, and age is most closely correlated with these measures. Above the age of 20 days, both wing wear and coloration show relatively little change and therefore are less useful indicators of age.

In a multiple regression analysis, a cubic polynomial model with separate slopes for males and females yielded a significantly better fit than a cubic polynomial model assuming a common slope ( $F = 4.8$ ,  $df = 3/657$ ,  $P < 0.05$ ) (Fig. 2), even when controlling for the size (head width) of the bees. The partial correlation between size and wing wear was significantly positive (partial  $t =$

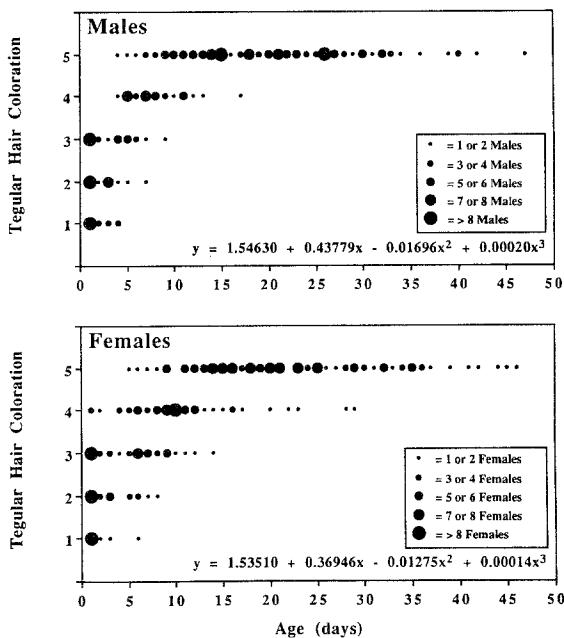


Fig. 3. Progression of tegular hair coloration over time for 55 male (top) and 91 female (bottom) *A. manicatum*. Hair coloration ranges from brightly orange (HC 1) over orange-brown (HC 3) to white (HC 5). Tegular hair coloration shows a significant nonlinear relationship with age ( $P < 0.0001$  for both sexes) but progresses significantly faster in males than in females ( $P < 0.005$ ).

2.57,  $df = 1$ ,  $P < 0.005$ ). Thus, wing wear increased faster in males than in females, increased more rapidly in larger individuals regardless of sex, but progressed most rapidly in large males and least rapidly in small females.

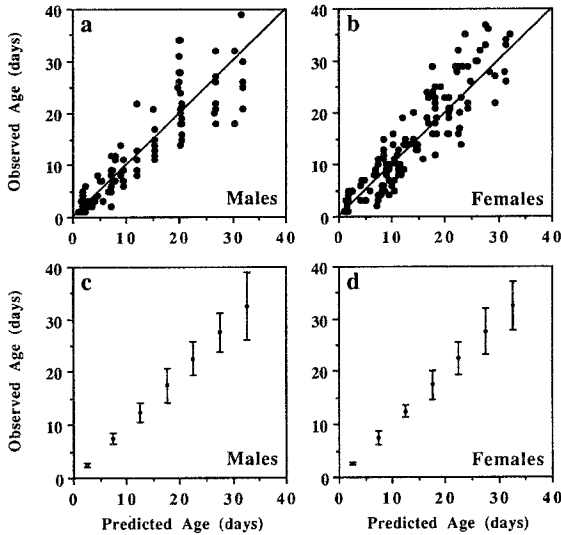
Similarly, tegular hair coloration progressed significantly faster in males than in females, as determined by a comparison of models fitting common versus separate slopes ( $F = 8.33$ ,  $df = 3/659$ ,  $P < 0.005$ ) (Fig. 3), and this effect was independent of size. Size itself showed no significant partial correlation with tegular hair coloration (partial  $t = -0.494$ ,  $df = 1$ ,  $P > 0.5$ ).

*Predicting Age from Wing Wear and Tegular Hair Coloration.* To test the accuracy of wing wear and hair coloration in predicting the age of bees, we divided the overall data set into two subsets, using one subset to derive multiple regression models that predicted age from field measurements, then applying this model to the other subset. Predicted age and actual age (as observed in the field) could then be compared for this second subset. Predictive models were based on repeated measures of wing wear, tegular hair coloration, and head width from randomly selected males ( $n = 35$ ) and females ( $n = 51$ ). Separate models were fitted for the two sexes. The best-fit model was derived by testing for significance of higher-order polynomials. To adjust for heteroscedasticity, the independent variable, age, was transformed logarithmically. Using this model, we then predicted the ages for each remeasurement of the 20 males and 40 females that had been set aside before derivation of the model and compared predicted ages with actual ages as observed in the field.

Multiple regression models including quadratic functions of wing wear and linear functions of both tegular hair coloration and head width provided the best fit for both the sample of 35 males and the sample of 51 females. Wing wear was the most important predictor of age (explaining 59.0% of the variance in males and 66.9% in females), followed by tegular hair coloration (explaining 28.2% of the variance in males and 10.3% in females) and by head width (explaining 3.1% of the variance in males and 4.1% in females).

The correlations between predicted age and observed age for males ( $r = 0.905$ ,  $P < 0.001$ ) and females ( $r = 0.925$ ,  $P < 0.001$ ) are presented in Figs. 4a and b. For both males and females, predictions are best met at ages lower than 15 days, but less so at higher ages. This pattern becomes more evident when calculating average differences between observed and predicted ages and their corresponding 95% confidence intervals for "cohorts" of individuals predicted to fall within 5-day age intervals (Figs. 4c and d). Below 15 days of age, predictions for such 5-day age "cohorts" are accurate to about  $\pm 1$  day; for ages above 15 days of age, predictions are accurate to  $\pm 3$  to 5 days.

In summary, wing wear and tegular hair coloration represent valid and reliable measures of age in the solitary wool-carder bee *Anthidium manicatum* (Figs. 2 and 3). The exact relationship between these measures and time, however, varies between the sexes and is also dependent on body size. First, holding



**Fig. 4.** Correlations between observed age and predicted age derived from wing wear and coloration measurements taken repeatedly on (a) 20 males ( $r = 0.905$ ) and (b) 40 females ( $r = 0.925$ ). Observed and predicted ages are most closely correlated at ages less than 15 days. Ninety-five percent confidence intervals of average residuals (difference between observed and predicted ages) for “cohorts” that are predicted to fall within 5-day age intervals are shown in c for males and in d for females. Confidence intervals indicate that predictions are accurate to about  $\pm 1$  day for ages less than 15 days and accurate to  $\pm 3$  to 5 days for ages above 15 days.

body size constant, males accumulate wing wear at a faster rate than females. The faster accumulation of wing wear in males is the likely consequence of male territoriality, involving constant patrolling and frequent fights with intruding conspecifics (Severinghaus *et al.*, 1981; Mueller, 1987; Wirtz *et al.*, 1988). Second, within each sex, larger individuals accumulate wing wear at a faster rate than females. The influence of body size on wing wear may be due to the increased mechanical stress experienced by wings of larger individuals (Danforth, 1989).

Of the two parameters examined, wing wear is a considerably more important predictor of age than tegular hair coloration, explaining twice as much of the variance in males and six times as much in females. Both measures, combined with head width, allow for a precise determination of average age for groups of bees predicted to belong to the same age cohort (Figs. 4c and d). For bees predicted to be less than 15 days old, age can be ascertained with confidence limits of about  $\pm 1$  day. Above 15 days of age, confidence intervals are wider

and span about  $\pm 3$  to 5 days. Confidence intervals therefore are narrow enough to provide a detailed picture of the age demography in populations surveyed with this method.

In principle, the above noninvasive method of age determination can be applied to other insect species showing gradual wing wear, fading of color, or any additional age-dependent morphological change. The prediction of age from such measures, however, will require species-specific or even population-specific calibration of the aging scale. Calibration may also be required for different seasons (e.g., if flight activity patterns vary between years) or for different castes in social species (e.g., if behavioral repertoires vary between workers and reproductives). Because of the necessary initial investment in calibration, the proposed noninvasive method will be most useful in long-term behavioral or ecological research. Finally, the finding that wing wear increases with body size suggests that the proposed method should provide more precise estimates of age in larger insect species, that is, species that accumulate wear at a faster and more constant rate. For allodapine and halictine bees, for example, Michener (1971) and Michener and Wille (1961) noted the lack of wing wear in minute species but appreciable wear in larger species.

### ACKNOWLEDGMENTS

We thank the staff of the Cornell Plantations for cooperation and permission to work in the flower gardens of Cornell University and B. Alexander, G. Eickwort, B. Danforth, and particularly W. Weislo for comments on the manuscript.

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