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VARIATIONS IN THE SIZE AND WEIGHT OF ADULTS
OF ORNITHODOROS MAROCANUS VELU (ACARI: ARGASIDAE),
ACCORDING NYMPHAL BLOOD-INTAKE

by Félix Fontanilla OLMEDO 1, Agustín ESTRADA-PENA 2, Joaquín Castellá ESPUNY 3

ABSTRACT: Variations in blood intake by individual nymph-4 specimens of O. marocanus influence the number of instars in the life cycle and generate significant deviations in the measurement and weight ranges in the adults. Fourth-instar nymphs may produce an additional nymphal instar when only limited blood resources are available. Male specimens derived from different nymphal instars, from partially engorged nymphs, or from already fed males, show significant differences in body measurements. Males and females also differ in the amount of blood ingested, according to the previous nymphal instar.


INTRODUCTION

Much confusion exists concerning the taxonomic status of the Ornithodoros erraticus complex of species. Currently, three species are recognized: O. erraticus (Lucas), O. marocanus Velu, and O. sonrai Sautet & Witowski (HOOGSTRAAL, 1985). Some authors, however, have claimed that only one species exists, and, following the rule of priority, established O. erraticus as the single name for a geographically widespread species (COLAS-BELCOUR, 1928; CHABAUD, 1954). OLEAGA (1989) mentioned that the three species included in the complex by HOOGSTRAAL (1985) produce fertile offspring in interspecific crossbreeding. However, the three species are still recognized in most recent works on African Swine Fever Virus epidemiology (ENDRIS et al., 1992). The question of variations in size and morphological features of some other body parts still remains. Because of the acknowledged role played by both O. erraticus and O. marocanus in the epidemiology of African Swine Fever Virus (SÁNCHEZ BOTÍA, 1963), taxonomic differentiation of the complex becomes a priority to confirm their status as significant vectors. However, the multiple life-cycle of argasids, with a variable number of nymphal stages, may generate great variability in morphological features of adults.

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The idiosomal size and weight of unfed and fed immature stages of *Hyalomma a. anatolicum* (Koch) are positively correlated, the larvae and nymphs showing a bimodal weight distribution (ARTHUR & SNOW, 1965). The same is true for some argasids, such as *Ornithodoros tholozani* (Laboulbene & Megnin) and *O. parkeri* Cooley (BALASHOV, 1972; POUND et al., 1986). However, the issue is confused in argasids because of their multi-nymphal developmental cycle. Thus variations in blood intake by individual nymphal specimens in *O. marocanus* may influence the instars’ number in the life cycle and generate significant deviations from the measurement and weight ranges in the adults. This paper aims to clarify whether there is a consistent relationship between the amount of blood ingested by nymphs of *O. marocanus* and subsequent moults, as well as divergence in adult sizes and weights, as a consequence of additional nymphal instars.

**MATERIAL AND METHODS**

The *Ornithodoros marocanus* colony used in this study was originally collected from a pig den in Valencia de Alcántara (Cáceres province, Spain). Larvae, first- and second-instar nymphs were fed on suckling laboratory mice; later nymphal and adult stages were fed on giant albino rabbits, anaesthetized by injection with ketamine. Nymphal instars were determined according to the methods of OLEAGA (1989). One hundred and twelve N4 (fourth nymphal stage) were used, 2 months after the N3-N4 moult. Each tick was individually fed on suckling white mice (5-8 days old), and forcibly detached after a given time interval. Partially engorged of fully replete N4 were arranged in 4 groups, according to their weight after the blood meal; with respective means of 25 %, 50 %, 75 % or 100 % of relative blood intake. Another group of 35 N4 was used as control, without feeding. Each tick was weighed immediately and 30 minutes after feeding, as a measure of coxal fluid weight. Ticks under study were observed daily, and were weighed and measured on the day of moulting. All the specimens were again fed to full repletion, one month after the previous meal, disregarding the percentage of blood intake in the previous feeding phase, under the same conditions previously described. ANOVA was routinely used in searching for statistically significant differences in size and weight between instars.

**RESULTS**

Table 1 includes data for the N4 moults, as the number of instars observed from the four different classes of blood intake. Specimens of the 25 % group produced 92.86 % nymphs (fed but not moulted), 3.6 % males and 3.6 % N5. Group 2 (50 % of blood intake) produced 50 % of non-moulted nymphs, 42.8 % of males and 7.1 % of N5. The N4 from classes 3 and 4 (75 % and full repletion, respectively) yield greater number of males (47.6 % and 62.9 %, respectively) and of N5 (23.8 % and 37.1 %). No N4-derived females were observed in any group of ticks. In figure 1, the average parameters for the weight of specimens, coxal fluid production and amount of blood ingested, amongst others, are included. These ‘intermediate’ (unmoulted N4) nymphs displayed a lower weight than that of males and N5, as well as lower figures for the amount of blood ingested, production of coxal fluid, and weight after moult. However, moulting time was similar for all the specimens. Weight of males ranged from 30 to 65 mg; the male-producing N4 ingested between 31 and 127 mg of blood. The average size of males obtained from N4 was 2.66-4.34 mm. N5 specimens weighed 26-62 mg, had a size of 2.17-4.76 mm and a blood intake between 52 and 168 mg. Unmoulted nymphal instars weighed 38-115 mg, with an average size of 2.59-3.43 mm, and a blood intake of 19-87 mg. Figure 2 includes the same parameters arranged by the blood intake class (1 : 25 %, etc.).

**Table 1**: Number and percentage (in parentheses) of specimens moulting to males (m) nymphs 4 (N4) or nymphs 5 (N5) according to the class of blood ingested (left column).

<table>
<thead>
<tr>
<th>Blood Intake</th>
<th>Males</th>
<th>Nymphae 4</th>
<th>Nymphae 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 %</td>
<td>1 (3.57)</td>
<td>26 (92.86)</td>
<td>1 (3.57)</td>
</tr>
<tr>
<td>50 %</td>
<td>12 (42.86)</td>
<td>14 (50.00)</td>
<td>2 (7.14)</td>
</tr>
<tr>
<td>75 %</td>
<td>10 (47.62)</td>
<td>6 (28.57)</td>
<td>5 (23.81)</td>
</tr>
<tr>
<td>Full Repletion</td>
<td>22 (62.86)</td>
<td>0</td>
<td>13 (37.14)</td>
</tr>
</tbody>
</table>
The values correspond well with the already mentioned observations. The weight of newly-moulted specimens is greater when derived from fully engorged nymphs, while moulting time was very similar for all the groups.

Table 2 displays the stages obtained from the different instars in the previous moulting period. Thus, N4 ticks produced 53.3 \% of females, 24.4 \% of males, and 22.2 \% of N4 again. Furthermore, N5 gave high numbers of females (80.9 \%) and low numbers of males (9.5 \%); none of N5 specimens moulted to a new nymphal stage, and two died during the experiment. Figure 3 includes the data observed for feeding parameters in the subsequent repletion period, sorted by the stage obtained after the first N4 molt. Although the repletion times were similar for the three stages (males, N4 and N5), the amount of blood ingested is clearly higher for both N4 and N5 instars. N5 derived males ranged between 3.36 and 3.99 mm in size and weighed 35-99 mg. Females obtained from N5 ranged between 2.17 and 4.76 mm, weighed from 62 to 142 mg;
however, N4-derived females (after a new blood ingest by unmoulted N4) were 3.85-4.62mm in size and weighed 63-126 mg.

There are no significant differences in the size and weight between N4- and N5-derived females (p = 0.906 and 0.886, respectively). However, ANOVA test shows very significant differences in the size (p = 0.0035) and weight (p < 0.0001) of males that undergo a short feeding period. Table 3 summarises data for the adults, in terms of the stage from which they moulted. Figures 4 and 5 display the frequency distribution of size and weight for the males at the end of experiment.

**DISCUSSION**

Our data show that males of *Ornithodoros marocanus* display significant differences in both size and weight according to the previous instar in their life cycle. When N4 are allowed only a small portion of the normal blood intake needed for repletion, an additional nymphal instar appears. Males moulting directly from fully-engorged N4 have different morphometries in comparison with those which moult from the additional nymph. Furthermore, females show little variation in both their size and weight following the same variations in the number of nymphal instars. Females may also be derived from previously-unmolted N4 specimens when a full blood intake is provided after the first, partial blood meal. In our experiments, no females were obtained directly from N4, although the latter were fully replete. This is in accordance with previous papers dealing with the life cycle of several argasid species. Fifth instar nymphs of both *Ornithodoros tholozani* and *O. parkeri* produced only adults,
FIG. 3: Parameters for the second feeding and moult phases, according to the preceding instar. Same abbreviations as for Fig. 1.

<table>
<thead>
<tr>
<th></th>
<th>pre-feeding</th>
<th>first feeding</th>
<th>second feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>males-1</td>
<td>2.17-3.22 (2.79)</td>
<td>2.80-3.68 (3.21)</td>
<td>2.66-4.34 (3.39)</td>
</tr>
<tr>
<td>males-2</td>
<td>2.66-3.08 (2.83)</td>
<td>2.80-3.15 (2.92)</td>
<td>3.36-3.99 (3.62)</td>
</tr>
<tr>
<td>females-1</td>
<td>2.45-3.08 (2.75)</td>
<td>2.94-3.71 (3.32)</td>
<td>2.17-4.76 (4.18)</td>
</tr>
<tr>
<td>females-2</td>
<td>2.45-3.08 (2.85)</td>
<td>2.73-3.43 (3.11)</td>
<td>3.85-4.62 (4.17)</td>
</tr>
</tbody>
</table>

TABLE 3: Minimum, maximum and average (in parentheses) size (top table, in mm) and weight (lower table, in milligrams) for males and females at the end of experiment, according to the number of previous stages. Males-1, specimens moulting as N4-male-male; males-2, specimens moulting as N4-N4male; females-1, specimens moulting as N4-N4-female; females-2, specimens moulting as N4-N5-females.
Fig. 4: Frequency distribution of size of males (in cm, X axis) after the second feeding and moulting period. Data at the right show the p-values for ANOVA between and within stages.

Fig. 5: Frequency distribution of weight (in milligrams, X axis) after the second feeding and moulting period. Data at the right show the p-values for ANOVA between and within stages.
mostly females (BALASHOV, 1972; POUND et al.,
1986). O. papillipes N4 produced 98 % adults and
O. parkeri N4 88 % adults, 79 % of which were
females. BALASHOV (1972) proposed that the body
size of nymphal argasids has an upper limit, beyond
which existence as an immature is impossible. He
reported prolonged development of Ornithodoros
tholozani, in which N6-N8 were only slightly larger
than N4 and N5. HAFEZ et al. (1971) reported that
nymphal Argas (P.) arboreus destined to become
males, ingested 77-80 % as much blood volume as
those which became females.

Various factors influence the number of nymphal
instars in argasid ticks, which is genetically control­
led. The genetic plasticity of each species, however,
may allow variation in the number of nymphal
instars, depending on several environmental and
host factors (POUND et al., 1986). However, no data
on adult morphological variation have been repor­
ted. The high number of strains in O. erraticus has
previously been reported by CHABAUD (1954), in a
study with specimens collected at several points
within its distribution area. Our data on male
body-size variation, depending on the previous
nymphal instar or coming from already fed males,
may introduce an additional bias in the systematic
determination of the O. erraticus complex of spe­
cies, if there is more than one species.

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REFERENCES

ARTHUR (D. R.) and SNOW (K.), 1966. — The significance
of the size in the immature stages of Ixodoidea. —
Parasitology 56: 391-397.

BALASHOV (Y. S.), 1972. — Bloodsucking ticks (Ixodoi­
dea) vectors of disease to man and animals. — (English
8: 161-376.

COLAS-BELCOUR, (J.), 1928. — Sur l’identité d’Orrnhodoro­
s erraticus Lucas et d’O. marocanus Velu. — Arch.

CHABAUD (A. G.), 1954. — Ornithodoros erraticus (Lucas,
Comp. 29: 89-130.

ENDRIS (R. G.), HESS (W. R.), CAIASO (J. M.), 1992. —
African Swine fever virus infection in the Iberian soft
tick Ornithodoros marocanus (Acari ; Argasidae). — J.
med. Entomol. 29 (5) : 874-878.

HAFEZ (M.), ABDEL-MALEK (A. A.), and GUIRGUIS
(S. S.), 1971. — The subgenus Persicargas (Ixodoidea,
Argasidae, Argas). 12. Biological studies on the imma­
ture stages of A. (P.) arboreus Kaiser, Hoogstraal &
Kohls in Egypt. — J. med. Entomol. 8: 421-429.

HOOGSTRAAL (H.), 1985. — Nuttallicellidae and Argasidae
as parasites and vectors. — Adv. Parasitol. 28:
101-185.

OLEAGA PEREZ (A.), 1989. — Distribución, biología y
relaciones de Ornithodoros erraticus con el ganado
porcino en España, en áreas enz06ticas de Peste
Porcina Africana. — Tesis Doctoral, Universidad de
Salamanca.

POUND (J. M.), CAMPBELL (J. D.), ANDREWS (R. H.) and
OLIVER (J. H.), 1986. — The relationship between
weights of nymphal stages and subsequent develop­
ment of Ornithodoros parkeri (Acari : Argasidae). — J.
med. Ent. 23 : 320-325.

SANCHEZ BOTIJA (A. C.), 1963. — Reservorios del virus
de la Peste Porcina Africana. Investigación del virus de
la P. P. A. en los artrópodos mediante la prueba de la
hemoadsorción. — Bull. Office Int. des Epizooties, 60:
895-899.