THE AGE-RELATED STRUCTURE OF SOME IXODIDE POPULATIONS FROM DIFFERENT GEOGRAPHIC AREAS IN NORTH-EAST AND SOUTH-EAST OF ROMANIA

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Abstract: The age-related structure of some ixodid populations from different geographic areas in north-east and south-east of Romania was released. The studies included the unfed and live nymphs and females of Ixodes ricinus and Dermacentor marginatus collected from environment by Nutall method. In both species, were detected, based on lipid reserves, four different age groups. The young age, with a big amount of lipids, was dominant in March, September and October, and the old age, without nutritional reserves, was found in summer and November. The study confirms the presence of two cohorts for each stadium one is active in spring, and the other one, in autumn, each of them with different mechanism of diapause, but starting and maximum of ticks activity depending on the local ecological factors (abiotic, especially microclimat, and biotic factors).

INTRODUCTION

The seasonal pattern of activity of questing ticks from temperate areas usually is bimodal, with two peaks: the first is high in spring and the second in autumn, much lower than first (Gray, 1982; Soneshine, 1991; Mitrea și col., 2001).

This bimodal pattern of seasonal tick activity generated numerous discussion and theories.

Nowadays, there are experimental studies which show that questing tick activity is coordinated by different mechanisms of diapause (behavioural diapause and morphogenetic diapause). According with these studies, the bimodal pattern of seasonal tick activity requires two cohorts of ticks of all stages, each of them with a different mechanism of diapause (Gray, 1982, 1991; Walker A.R., 2001). These subpopulations could be detectable by method of grading of ticks into groups with different physiological ages (according to the method described by Gray, 1987, and Uspensky, 1995).

Physiological age of ixodid ticks is determined by estimating of reserves of lipids and proteins derived from the previous blood meal; ixodid use these reserves during the potentially long questing period. The level and status of lipid reserves in unfed ticks has been proposed as an index of the physiological age. The dynamics of the use of these substances reflect tick age (Uspensky L, 1995).

The objective of this study was to establish the age-related structure of some ticks populations (of Ixodes ricinus and Dermacentor marginatus) from different geographic areas in north-east and south-east of Romania, in order to explain the seasonal pattern activity
MATERIALS AND METHODS

In order to age grading of ticks populations, initially the physiological age of ticks was determined, based on lipids reserves. Lipids reserves were emphasized in histological sections performed at the freezing microtome, and stained with Sudan red (according to Razumova (1977) method, [the results was published in a prior paper to this (Ionita M. and col., 2005)]. The registered results were interpreted based on graphical models, emphasizing the monthly prevalence of each age group. Establishing the physiological age groups was the bases for explanation of the annual dynamic diagram of studied ixodid populations.

The study was performed during 2001-2002, on unfed nymphs and adults of *Ixodes ricinus* and *Dermacentor marginatus*, collected from field (different geographic areas of Romania: north-east –Suceava county, two locations; south-east – Teleorman county, one location). Ticks were collected once per month (excepting December, January and February) from each location, using Nutall method (*dragging* or *flagging* cloths); we used a cloths in white cotton, 80x160 cm, held with bars along leading (like a *flag*) (Al. Niculescu and Tr. Lungu, 1968; Conroy, 1996). The flag was dragged on the surface of the pasture, over 10 m, slowly, and after that, examined. All ticks attached were *in situ* counted, and collected into tubes with damp paper and transported live to the laboratory for identification. After identification, the samples were prepared for freezing-section and stained with Sudan red, in order to emphasize lipids reserves (Ionita M. si col., 2005).

RESULTS AND DISCUSSION

Microscopic examination of tick slides obtained by freezing-section and stained with Sudan red showed significant differences of lipids reserves, depending on collecting moment, for both species (*Ixodes ricinus* and *Dermacentor marginatus*), and for both adult and nymphs. According to the amount of lipid reserve, we have distinguished 4 main physiological age-groups (tabl 1):

- age-group 1 (young group): a major amount of lipid;
- age-group 2 and 3: moderate lipids reserves;
- age-group 4 (old group: no lipid reserves.

Cumulative data about age related structure of the both species populations are summarized in table 1.

These results showed that, both in nymphs and adults populations, the young group (age group 1 – with major lipid reserves) is prevalent in March, and the old group (age group 4 – no lipid reserve) are prevalent in summer (July, August). These data are in according with similar studies that emphasize that for he most ticks moulting process was released during of springer months, when environmental conditions become favourable (average of temperature over 10˚C). Presence of ‘old’ samples (age group 4), in adults population, in earlier spring (March) (fig. 2) shows that these proceed from nymphs which were moulted in autumn of the previously year ant they had hibernated as adult. This aspect is also mentioned in some specialty literature that assert these species can hibernate in all instars (Cernăianu, 1957; Sonseshine, 1991).
Table 1 Age related structure of nymphs and adults of some ixodid populations of *Ixodes ricinus* and *Dermacentor marginatus* (cumulative data for both species, for two years: 2001, 2002)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age group</th>
<th>Monthly prevalence</th>
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<td>Nymph</td>
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<td>Nymph</td>
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Also, it has been ascertained the prevalence of the young group (age group 1) in sample collected in September and October, both in adults and nymphs, but their prevalence was lower in the next month.

Data analyzing emphasized a very similar structure of populations of adult and nymphs, with two cohorts for each, a cohort being active in spring, and one in autumn.

Spring cohort started in March, for both instars, when the average of air temperature was over 10°C (fig. 3-6), and autumn cohort started in September when the environmental conditions became again favourable. Both spring and autumn cohorts ‘get old’ in the next months, so in the middle of summer (July) and the middle of the cold season (November) the old group (age group 4) has a bigger prevalence.
Figure 1
Age related structure of nymphs: monthly prevalence of different age group
(cumulative data for both species, for two years: 2001, 2002)

Figure 2
Age related structure of adults: monthly prevalence of different age group
(cumulative data for both species, for two years: 2001, 2002)
The results of this study confirm hypothesis with two annual cohorts, spring and autumn cohorts, each of them with different mechanism of diapause (Gray, 1991), but starting and maximum of activity are depending on the local ecological factors (abiotic, especially microclimat, and biotic factors).

Evidence for these two mechanism of diapause has been documented by Belozerov (1967, 1971a,b, 1982), Gray (1985, 1987), Walker A.R. (2001). They ascertain that spring cohort has a behavioural diapause, but autumn cohort has a morphogenetic diapause. Behavioural diapause occurs in ticks after their moult, so their potential questing activity is suppressed. Morphogenetic diapause occurs in ticks which have recently fed and are about to develop to the next instar, suppressing their potential development.

In our study, based on corroborated results of physiologic age-grading, numeric abundance and features of microclimat, it should draw the conclusion that in ticks population from north-east areas (Suceava county), with a microclimat more inclement, severe in winter, probably the behavioral diapause is predominant, because this one permits performing of oviposition and moult in the most favourable microclimate (spring); the pattern of seasonal activity of ticks from this area showed a high peak in spring, and the autumn peak was very low or even absent (fig. 3, 4).

In areas from south-east (Teleorman county), where there is a microclimate more mild than in north-east, which permit survival of different instar over winter, should be possible both types of diapause, hypothesis asserted by the other authors, too (Gray, 1991). This could be an explanation of the biphasic pattern of questing activity of tick populations from this area. The diagram of annual dynamic shows a high peak in spring and an other one in autumn, the autumn peak representing at least 25% from the maximum of spring (fig. 5, 6).

The biphasic seasonal dynamic of ticks, with involving of two annual subpopulations (cohorts), emphasizes the survival advantage of these arthropods with complex life cycle, allowing that the major biological processes (eating, moult, oviposition) to occur in the most favourable environmental conditions, and the different mechanisms of diapause assure their survival in unfavourable conditions. As conclusive point, we can say that the microclimate has a determinant role on ticks activity.

CONCLUSIONS

1. In this study it was established the age related structure of some populations of *Ixodes ricinus* and *Dermacentor marginatus* (adults and nymphs collected from field) from different geographical areas from North-East and South-East of Romania.

2. According to the amount of lipid reserve, we have distinguished 4 main physiological age-groups, for both species and both instars (nymphs and adults), the prevalence of each age group depending on collected moment.

3. The ‘young group’ (age-group 1), with a major lipid amount, was predominant in March (65% for adults, and 75% for nymphs), also in September (62,85% - for adults and 66,66% for nymphs) and October (45,7% for adults and 40% for nymphs); during of the summer and November months ‘the old’ group (age-group 4, no lipid reserves), was predominant.

4. The results of this study confirm theory with two annual cohorts, spring and autumn cohorts, each of them with different mechanism of diapause, but starting and maximum of activity are depending on the local ecological factors (abiotics, especially microclimate, and biotic factors); this theory could be an explanation of the biphasic pattern of questing activity of tick populations from this area.
Figure 3. Annual dynamic (2001) of *Ixodes ricinus* populations from North-East of Romania, in relation with temperature variation

Figure 4. Annual dynamic (2002) of *Dermacentor marginatus* populations from North-East of Romania, in relation with temperature and precipitations variation

Figure 5. Annual dynamic (2001) of *Ixodes ricinus* populations (from South–South-East) in relation with temperature and precipitations variation

Figure 6. Annual dynamic (2002) of *Dermacentor marginatus* (from South-South East of Romania) in relation with temperature and precipitations variation
BIBLIOGRAPHY


