Desiccation Tolerance of the Tardigrade Milnesium tardigradum Collected in Sapporo, Japan, and Bogor, Indonesia

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Desiccation Tolerance of the Tardigrade *Milnesium tardigradum* Collected in Sapporo, Japan, and Bogor, Indonesia

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ABSTRACT—A tardigrade *Milnesium tardigradum* showed anhydrobiotic capacity, in which the desiccation tolerance, given by the mean survival rate under desiccation at different relative humidity levels, was significantly higher in the Sapporo (Japan) population than that in the Bogor (Indonesia) population. Accordingly, the surviving tardigrades took a significantly longer time for revival in Bogor than those in Sapporo. The higher tolerance of the Sapporo population is thought to be related to the low relative humidity and low temperature such that the animals experience 41% RH in May and often –10°C or lower in winter.

Key words: desiccation tolerance, anhydrobiosis, Tardigrada, *Milnesium*, intraspecific variation

INTRODUCTION

Tardigrades are known to distribute over many areas of the world, from sea to terrestrial environments. However, even in terrestrial habitats, they are in need of ambient water for foraging, reproduction and other activities, and therefore, all tardigrade species are regarded as aquatic animals. Once the surrounding water of such species has evaporated, many species, particularly those of which are terrestrial, enter anhydrobiosis, defined as an ametabolic state induced by desiccation and followed by revival when rehydrated (Keilin, 1959). Anhydrobiosis of tardigrades has been frequently studied for many years in relation to its physiological and biochemical aspects (Crowe and Cooper, 1971; Wright *et al*., 1992; Wright, 2001), but rarely in an ecological sense.

Wright (1989) had compared desiccation tolerance among seven tardigrade species and detected their interspecific variations reflecting on the difference of their habitat conditions (Wright, 1991). Furthermore, Jönsson *et al*. (2001) made the same comparison between Italian and Swedish populations of *Richtersius coronifer* and *Ramazzottius oberhauseri*, but they could not detect any significant intraspecific variation, partly because their experiment was conducted under a single condition of desiccation treatment, i.e. only 65% relative humidity (RH).

In this study, we tested the intraspecific variation of desiccation tolerance by comparing survivorships of tardigrades which were collected in Sapporo, Japan, and Bogor, Indonesia, and desiccated under several conditions of RH.

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MATERIALS AND METHODS

Study sites and materials

Monthly mean temperature of 2003 ranges from 22.1°C (June and September) to 24.7°C (March) in Bogor, and that of Sapporo from –3.6°C (February) to 21.3°C (August) where the minimum temperature is often –10°C or lower. Monthly minimum RH ranges from 77% (June) to 95% (April) in Bogor and 41% (May) to 61% (August) in Sapporo in the same year.

The tardigrade *Milnesium tardigradum*-inhabited lichens were collected from trees in Bogor and Sapporo on 1 October and 5 October of 2003, respectively. The dry lichens were kept in the laboratory until experiments were initiated on 7 October 2003. The lichens were immersed in distilled water for 10 to 24 hours and then the active animals in the water were collected via pipette, and they were immediately used for experimentation within 30 minutes. All experiments were completed by 23 January 2004.

Experiment for desiccation tolerance

A single drop of distilled water containing 10 animals was put on a watch glass in a tightly closed desiccator floored with 20 ml KOH solution of various concentrations in order to control RH levels from 50 to 62%, increasing by 2%, in accordance to Solomon (1951). The laboratory temperature was maintained at 25°C as RH levels in the desiccator were confirmed using a hygrometer probe connected to a two channel logger (SK-L200TH, SATO). The experiment was commenced once the water surrounding animals had visually disappeared due to evaporation. The animals were desiccated for 1 hour, then removed from the desiccator and immediately rehydrated by 0.5 ml of distilled water. After rehydration, samples were observed under a stereomicroscope, and, whenever locomotor rhythms of the lobopodia were confirmed, such an animal was removed with a pipette and judged as “revival”. On the other hand, when the animals did not show the rhythms within a period of 3 hours, they were judged as “death”. Four replicates were performed for each RH case. Values for survival rate percentage have been

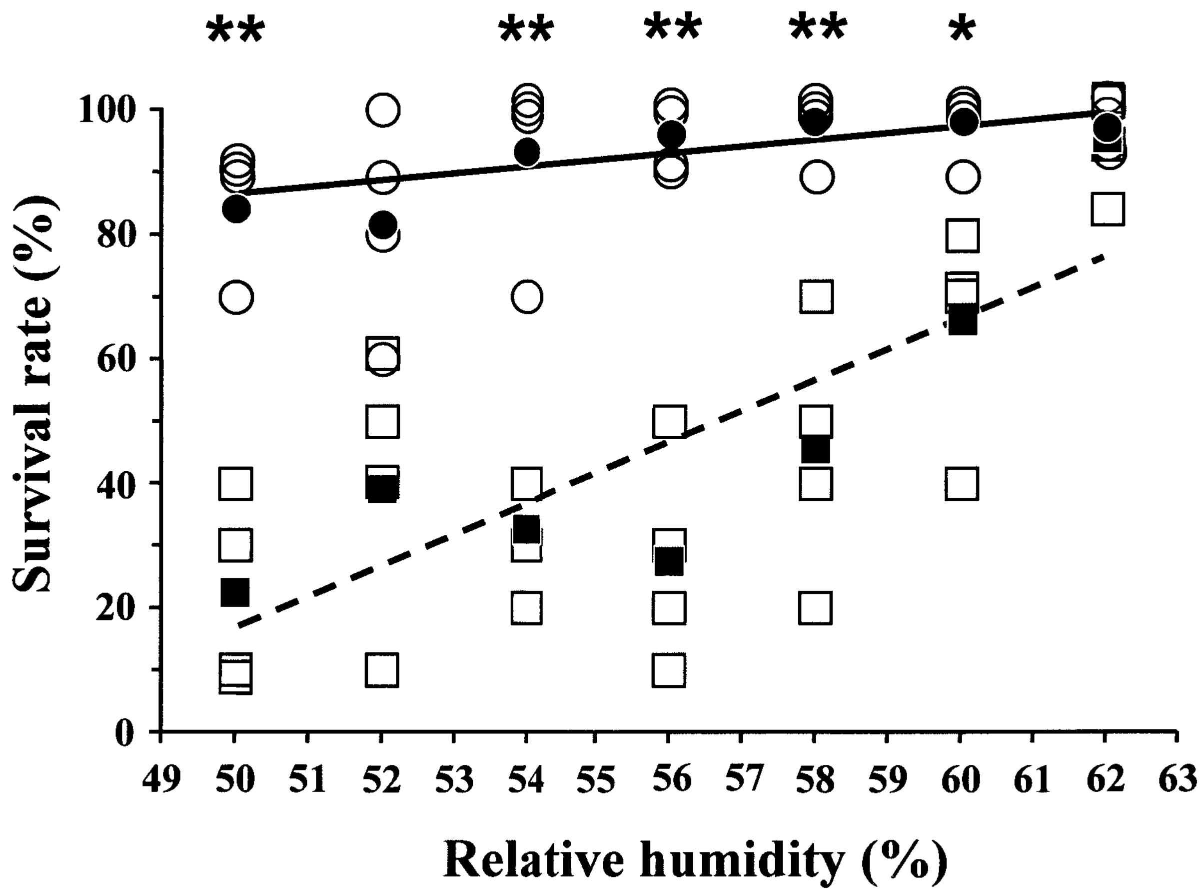

Fig. 1. Survival rates of *M. tardigradum* from Sapporo (○) and Bogor (□) after 1 h desiccation at varying relative humidity (25°C). Each replicate consisted of 10 animals. Correlation between RH and survival rate was significant in Sapporo (solid line; r^2=0.197, P<0.05) and in Bogor (broken line; r^2=0.524, P<0.0001). Mean survival rates are shown by ● for Sapporo and ■ for Bogor. Overall difference of the mean survival rate between the two populations was significant (Two-way ANOVA, P<0.0001). Asterisks denote significant differences of mean survival rates between the two populations (Bonferroni's multiple comparison test; *: P<0.05; **: P<0.0001).

arc sine transformed before ANOVA procedures were undergone.

RESULTS

The mean survival rate ranged from 82.5% at 52% RH to 97.5% at 58 and 60% RH in the Sapporo population and from 22.5% at 50% RH to 92.5% at 62% RH in the Bogor (Fig. 1). Results from a two-way ANOVA detected a significant difference in mean survival rates between the two populations ($F_{1,\ 42}$=125.485; P<0.0001). Bonferroni's multiple comparison test showed a significant inter-population difference at 50%, 54%, 56%, and 58% RH (all with P<0.0001), and 60% RH (P<0.05), indicating that the survival rate of the Bogor population was as high as that of the Sapporo population under the condition of high humidity.

Regression lines of the two populations shown in Fig. 1 display the existence of a significant correlation between RH and survival rate in both Bogor (coefficient of determination, r^2=0.524, P<0.0001) and Sapporo (r^2=0.197, P<0.05). Thus, the survival rate decreased remarkably under the low relative humidity in the Bogor population.

Fig. 2 represents the revival times of each individual, which had survived in the desiccation tolerance test. The mean revival time ranged from 14.1±4.4 min at 62% RH to 39.3±33.4 min at 50% RH in the Sapporo population, but from 18.1±6.5 min at 62% RH to 62.3±38.2 min at 50% RH in the Bogor population. This difference implies that tardigrades of Bogor took a longer time to revive from anhydrobiosis. Although the correlation between RH and revival time was significant in Sapporo and Bogor (both with P<0.0001), r^2 was larger in Bogor (0.361) than that in Sapporo (0.155), suggesting that levels of RH were more effective to revival time in Bogor.

DISCUSSION

Unlike *R. coronifer* and *R. oberhauseri*, which had not shown any intraspecific variation of anhydrobiotic survival (Jönsson *et al*., 2001), *M. tardigradum* showed a significant variation of desiccation tolerance. In nematodes *Steinernema feltiae* (Solomon *et al*., 1999) and *Heterodera avenue* (Williams, 1978), as well as *M. tardigradum*, desiccation tolerance is thought to be adaptive as it was theoretically considered by Jönsson and Järemo (2003). Since storage

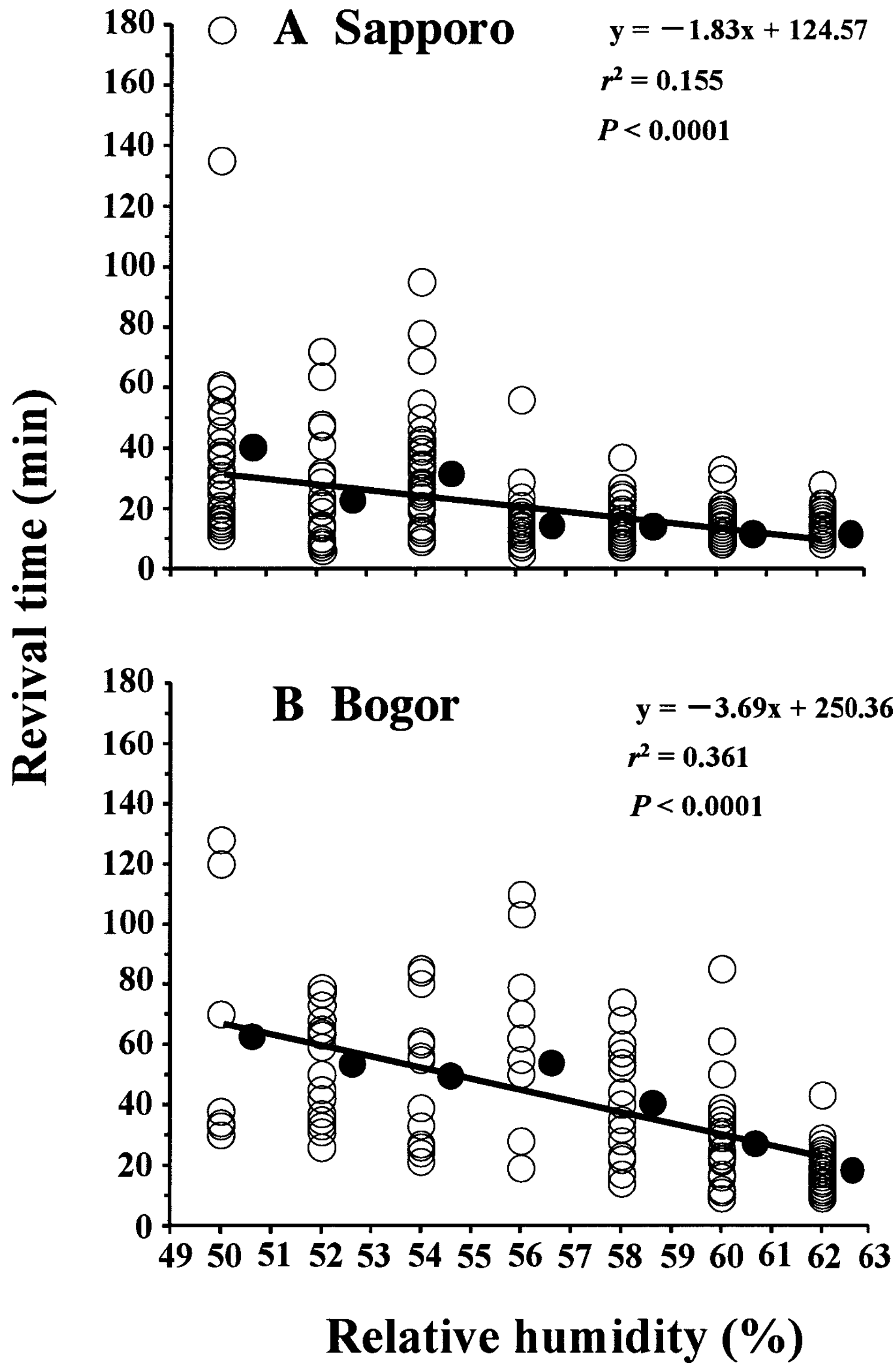

Fig. 2. Revival times of *M. tardigradum* which survived in the corresponding experiment from Fig. 1. The revival time of each individual is shown by ○. The mean revival time, represented by ●, is significantly correlated with relative humidity in both Sapporo and Bogor.

cells are significantly smaller in post-anhydrobiosis than those in pre-anhydrobiosis, the anhydrobiosis requires a large amount of energy (Jönsson and Rebecchi, 2002). Furthermore, Jönsson and Järemo (2003) stated that the high anhydrobiotic capacity is preferred in regions which are always dried out. Since RH is lower in Sapporo than that in Bogor, the intraspecific variation of desiccation tolerance seems to be adaptive in *M. tardigradum*.

In addition to RH, the air temperature is lower in Sapporo than that in Bogor, in which the minimum temperature is lower than −10°C in winter. The higher tolerance to desiccation may be partly attributed to the low-temperature adaptation in Sapporo, due to the mechanism of anhydrobiosis being similar to that of cryobiosis (cryptobiosis induced by low temperature) (Wright, 2001). For instance, a nematode *Panagrolaimus davidi* shows “cryoprotective dehydration” when its surrounding water had been frozen at high subzero temperature or under a slow cooling rate (Wharton *et al*., 2003). The synthesis and accumulation of disaccharide trehalose have been reported in anhydrobiosis of *R. coronifer* (Westh and Ramløv, 1991) and in cold preacclimation of nematodes *S. feltiae*, *S. carpocapsae* and *S. riobrave* (Grewal and Jagdale, 2002). The biochemical reaction proceeded successfully under wet conditions (Westh and Ramløv, 1991), and the outcome of a high survival rate of tardigrades desiccated under high RH is thought to be due to this successful process.

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