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APPLICATIONS OF THE BOOTSTRAP TO INSECT PHYSIOLOGY

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ABSTRACT

The effect of temperature on development and the effect of photoperiod on diapause incidence of insects are common topics in insect physiology. Related to these topics, are 3 important concepts: the lower and upper developmental thresholds, which represent the lower and upper limits of thermal range for insects to develop, and the critical photoperiod, which causes diapause in 50% of a population. To compare lower or upper developmental thresholds of 2 different developmental stages or of 2 populations at the same developmental stage is difficult because of the lack of a suitable statistical method. Shi et al. (2010) proposed 2 methods for testing whether there is a significant difference between 2 lower developmental thresholds. However, these 2 methods are only applicable to the linear relationship between developmental rate and temperature. There are also many studies on the geographic variation in the critical photoperiods for different populations of an insect species. Also, a method is lacking for testing whether there is a significant difference between 2 critical photoperiods. In this study, we test bootstrap to determine if there is a significant difference between different parameters. Bootstrap can then be used to compare any 2 lower or upper developmental thresholds, or to compare 2 critical photoperiods. It can also provide the confidence interval of a critical photoperiod.

Key Words: developmental rate, diapause incidence, lower developmental threshold, upper developmental threshold, critical photoperiod, confidence interval

RESUMEN

El efecto de la temperatura sobre el desarrollo y el efecto del fotoperiodo sobre la incidencia de la diapausa de insectos son temas comunes en la fisiología de insectos. En relación a estos temas, hay tres conceptos importantes: los umbrales inferiores y superiores de desarrollo, que representan los límites inferiores y superiores del rango térmico de los insectos a desarrollar, y el fotoperiodo crítico, que causa la diapausa en el 50% de la población. Para comparar los umbrales inferiores y superiores de desarrollo de dos poblaciones en el mismo estado de desarrollo es difícil debido a la falta de un método estadístico adecuado. Shi et al. (2010) propusieron dos métodos para comprobar si existe una diferencia significativa entre los dos umbrales más bajos de desarrollo. Sin embargo, estos dos métodos sólo son aplicables a la relación lineal entre la tasa de desarrollo y de la temperatura. También hay muchos estudios sobre la variación geográfica en el fotoperiodo crítico para diferentes poblaciones de una especie de insecto. Además, también hace falta un método para comprobar si existe una diferencia significativa entre los dos fotoperiodos críticos. En este estudio, se utilizó el método de "bootstrap" para determinar si existe una diferencia significativa entre los diferentes parámetros. Se puede usar este método para comparar cualquier dos umbrales de desarrollo inferiores o superiores, o para comparar dos fotoperiodos críticos. También puede proveer un intervalo de confianza de un fotoperiodo crítico.

The effect of temperature on developmental time (d) and the effect of photoperiod on diapause incidence (%) are common topics of insect physiology. Developmental rate, the reciprocal of developmental time for completing a developmental stage, is a linear function of constant temperature over the mid-temperature range (Fig. 1). However, the relationship between developmental rate and temperature is nonlinear over the whole range including the low, mid, and high temperatures in which an insect species can develop (Fig. 2). There are 2 intersec-
tions between the developmental rate curve and x-axis: the lower developmental threshold below which development terminates, and the upper developmental threshold above which development also terminates. Rijn et al. (1995) suggested that the lower developmental thresholds of all developmental stages of an insect species should be constant, which was referred to as the rate isomorphy hypothesis by Jarosík et al. (2002, 2004). Shi et al. (2010) proposed 2 methods of testing whether there is a significant difference between 2 lower developmental thresholds, but these methods were only applicable to the linear relationship between developmental rate and temperature over the mid-temperature range. The comparison between 2 upper developmental thresholds is less well studied. The effects of temperature on development of different developmental stages for an insect species, and the comparison on effects of temperature on development of different geographic populations of an insect species have received much attention (e.g., Tauber et al. 1987; Stacey and Fellowes 2002; Gotô et al. 2010). However, surprisingly, comparing the lower or upper developmental thresholds has been neglected probably because of the lack of a suitable statistical method.

For many insect species, there is a critical photoperiod that can induce diapause in 50% of a population. The critical photoperiod is an important concept of insect physiology, and the variation of critical photoperiods among different geographic populations for an insect species has also gained much attention (e.g., Ankersmit & Adkison 1967; Tauber & Tauber 1972; Hong & Platt 1975; Gomi 1997; Ishihara & Shimada 1999; Ito & Nakata 2000; Kurota & Shimada 2003; Sun et al. 2007). However, a suitable statistical method of testing whether there is a significant difference between critical photoperiods of two geographic populations is also lacking.

The bootstrap (Efron & Tibshirani 1993; Davison & Hinkley 1997) is known for estimating the standard error and confidence interval for life table parameters and temperature thresholds. However, few entomologists have paid attention to its function of parameter comparison. In this study, we introduce the confidence interval based on bootstrap percentiles, and show how to use it to compare 2 lower or upper developmental thresholds, or 2 critical photoperiods.

**PRINCIPLE OF USING BOOTSTRAP TO COMPARE 2 PARAMETERS**

Suppose the transformation \( \hat{\theta} = m(\hat{\theta}) \) perfectly normalizes the distribution of \( \hat{\theta} \):

\[ \hat{\theta} \sim N(\theta, \sigma^2) \]

for some standard deviation \( \sigma \). Then the percentile interval based on \( \hat{\theta} \) equals (Efron & Tibshirani 1993):

\[ [m^{-1}(\hat{\theta} - z_{1-\alpha}c), m^{-1}(\hat{\theta} - z_{\alpha}c)] \]

where \( z_{1-\alpha} \) is the 100(1-\( \alpha \))th percentile of a standard normal distribution; \( z_{\alpha} \) is the 100 \( \alpha \)th percentile of a standard normal distribution; 0 < \( \alpha \) < 1. \( z_{0.025} = 1.960, z_{0.975} = 1.645, z_{0.841} = 1.000, \) etc.

Assume that a linear or non-linear model \( y = f(x) \) has several parameters. If we are interested in one of these parameters in this model, let \( q \) represent this parameter. Assume that there are 2 datasets, e.g., the temperature-dependent developmental rates of 2 stages. Then we use \( y = f(x) \) to fit these 2 datasets, respectively. The 2 fitted values of \( q \) can be obtained. Now we test whether there is a significant difference between these 2 values of \( q \). We resample the given dataset \( (x_i, y_i) \) \((i = 1, ..., n)\) with replacement to obtain \((x'_i, y'_i) \) \((i = 1, ..., n)\) \((j = 1, ..., B)\), where \( B \) represents the resampling times. Using \( f(x') \) to fit \((x'_i, y'_i)\), we obtain the fitted values of \( q'_j \) \((j = 1, ..., B)\). Because there are 2 datasets, we can obtain \( q'_1 \) and \( q'_2 \). Let \( D = q'_1 - q'_2 \). We can now determine whether there is a significant difference between 2 values of \( q \) by checking the confidence interval of \( D \) to determine if it includes 0. If 0 is included by this interval, there is no significant difference between these 2 values of \( q \); if 0 is not within this interval, there is a significant difference between these 2 values of \( q \). In this study, we use the 95% confidence interval.
APPLICATIONS

Comparing Two Lower Developmental Thresholds.

The following equation is widely used to describe the temperature-dependent developmental rates at a specified developmental stage:

\[ y = a + bx \]  

Here, \( y \) is developmental rate; \( x \) is temperature; \( a \) and \( b \) are parameters to be fitted. Let \( t \) represent the lower developmental threshold, and let \( k \) represent the sum of effective temperatures required for completing a specified developmental stage. We have

\[
\begin{align*}
\hat{t} &= \frac{\hat{a}}{\hat{b}} \\
\hat{k} &= \frac{1}{\hat{b}}
\end{align*}
\]  

Here, a symbol with a hat denotes the estimate of what this symbol represents. The estimates of \( a \) and \( b \) can be obtained from some textbooks of statistics (e.g., Xue & Chen, 2007). Campbell et al. (1974) provided the standard error formula for the estimates of \( t \) and \( k \):

\[
\text{SE}(\hat{t}) = \frac{\hat{b} \cdot \text{MSE} + (\text{SE}(\hat{b}))^2}{n \cdot \hat{y}^2} \]
\[
\text{SE}(\hat{k}) = \frac{\text{SE}(\hat{b})^2}{\hat{b}^2}
\]  

Here, MSE represents the mean squared error. It equals \( \sum (y_i - \hat{y})^2 / (n - 2) \), and \( n \) is the sample size.

There are 2 basic resampling schemes for regression models (Davison & Kuonen 2002): 1) resampling cases \((x_1, y_1), ..., (x_n, y_n)\), under which the bootstrap data are \((x_1, y_1'), ..., (x_n, y_n')\), taken independently with equal probabilities \(1/n\) from the \((x_i, y_i), \text{ and } 2) \text{ resampling residuals. Having obtained estimates } \hat{a} + \hat{b}x \text{, we take } \epsilon_i \text{ randomly from centered standardized residuals } \epsilon_1, ..., \epsilon_n \text{ and set } y_i = \hat{a} + \hat{b}x_i + \epsilon_i, \ i = 1, ..., n. \text{ The second scheme is more efficient than resampling pairs if the model is correct. In this study, we use the second scheme to resample the raw temperature-dependent developmental rate dataset of } \textit{Colaphellus bowringi} \text{ Baly (Coleoptera: Chrysomelidae) from 16 to 26 °C (Fig. 1) in 2 °C increments (Kuang et al. 2011). The lower developmental thresholds and sums of effective temperatures of the egg, larval and pupal stages are calculated by equations 2 and 3, and by bootstrap (Table 1). The 95% confidence intervals of the difference between any 2 lower developmental thresholds based on bootstrap percentiles are:

- Egg-larva: [-0.4797, 0.2776]
- Egg-pupa: [-0.3173, 0.4934]
- Larva-pupa: [-0.2253, 0.6093]

We can find that all the 95% confidence intervals of the differences between any 2 lower developmental thresholds include 0. Thus, there are no significant differences among the lower developmental thresholds at the egg, larval and pupal stages. It further demonstrates the rate isomorphy hypothesis. However, all the 95% confidence intervals of the differences between any 2 sums of effective temperatures do not include 0. Thus, there are significant differences among the sums of effective temperatures of 3 developmental stages.

It is necessary to point out that Ikemoto & Takai (2000) proposed another linear model for describing the effect of temperature on developmental rates at a specified developmental stage:

\[ y = \hat{a} + \hat{b}x + \epsilon_i \]

This model is more efficient than the previous one in terms of computational time and accuracy. It is recommended for future studies.

### TABLE 1. ESTIMATED LOWER DEVELOPMENTAL THRESHOLDS AND THE SUMS OF EFFECTIVE TEMPERATURES FOR \textit{C. bowringi}.

| Equations 2 and 3 | Bootstrap |
|-------------------|-----------|
| Egg | Larva | Pupa | Egg | Larva | Pupa |
| \( \hat{t} \) | 10.13 | 10.24 | 10.06 | 10.13 | 10.24 | 10.05 |
| SE \( (\hat{t}) \) | 0.12 | 0.15 | 0.16 | 0.12 | 0.15 | 0.16 |
| \( \hat{k} \) | 79.88 | 154.02 | 61.53 | 79.89 | 154.04 | 61.60 |
| SE \( (\hat{k}) \) | 0.87 | 2.17 | 0.92 | 0.85 | 2.09 | 0.92 |

\( \hat{t} \) represents the estimate of lower developmental threshold.  
\( k \), represents the estimate of sum of effective temperatures required for completing a specified developmental stage.
tal rate. This model shows many advantages relative to equation 1 (Miller 2011). Bootstrap can be also used to compare the lower developmental thresholds estimated by the new model of Ikemoto & Takai (2000). Here, we do not exhibit that.

Comparing Two Upper Developmental Thresholds.

There are many non-linear models for describing the temperature-dependent developmental rates (e.g., Logan et al. 1976; Sharpe & DeMichele 1977; Schoolfield et al. 1981; Taylor 1981; Wang et al. 1982; Lactin et al. 1995; Brière et al. 1999; Ikemoto 2005, 2008; Shi et al. 2011). In practice, each model has its advantage relative to others for different species of insects. In this study, we do not question which one is best. We only choose one to show the function of bootstrap in comparing any 2 upper developmental thresholds. Logan model (Logan et al. 1976) is often used to calculate the upper threshold (e.g., Bonato et al. 2007, Eliopoulos et al. 2010):

$$y = \psi \left[ \exp(\rho x) - \exp\left(\rho T_U - \frac{T_U - x}{\delta}\right) \right]$$  \[4\]

Here, $y$ is developmental rate; $x$ is temperature; $T_U$ is the upper developmental threshold; $\psi$, $\rho$, and $\delta$ are constants. Gotoh et al. (2010) reported differences in temperature-dependent development among 7 geographic strains of *Tetranychus evansi* Baker et Pritchard (Acari: Tetranychidae) from 15 to 40 °C in 2.5 °C increments. In this study, we only test if there is a significant difference (Fig. 2) between the first 2 strains (i.e., BP and FT strains, see Gotoh et al. [2010] for details). In general, mean or median developmental rates are used to carry out a non-linear fitting (e.g., Schoolfield et al. 1981; Ikemoto 2005, 2008; Shi et al. 2011). We cannot conclude that Logan model is absolutely correct, so the first schedule (i.e., resampling pairs) is used to do bootstrap. We use the nlinfit function of Matlab 6.5 (http://www.mathworks.com/) to perform the non-linear fitting. The fitted upper developmental thresholds of BP and FT strains are equal to 44.55 and 44.89 °C, respectively. The 95% confidence intervals of the difference between these 2 upper developmental thresholds of BP and FT strains based on bootstrap percentiles are:

$$\text{BP-FT} \quad [-6.74, 6.75]$$

Because 0 is included in this interval, there is no significant difference between these 2 upper developmental thresholds of BP and FT strains.

Comparing Two Critical Photoperiods.

It is known that photoperiod has an important influence on diapause incidence for many insect species. Some investigators have attempted to model such an effect (e.g., Kroon et al. 1997; Kurota & Shimada 2003; Timer et al. 2010). These models are useful in studying insects. In this study, we suggest using a non-parametric fitting method of loess, which is short of local regression (Cleveland 1979; Cleveland et al. 1991), to determine the effect of photoperiod on diapause incidence. A non-parametric fitting method does not consider the potential mechanism of the photoperiod-independent diapause, but it can in general fit the dataset very well. Thus, loess has more flexibility than a parametric model. We also use loess to predict the critical photoperiod. Then we test whether there is a significant difference between 2 critical photoperiods of 2 geographic strains (Fig. 3) of *Bruchidius dorsalis* Fahraeus (Coleoptera: Bruchidae) (Kurota & Shimada 2003). The predicted critical photoperiods of Tatsuno and Sagamihara strains are 11.74 and 12.07 h, respectively. The 95% confidence intervals of the difference between these 2 critical photoperiods of Tatsuno and Sagamihara strains based on bootstrap percentiles are:

$$\text{Tatsuno-Sagamihara} \quad [-0.9270, 0.4766]$$

Because 0 is within this interval, there is no significant difference between these 2 critical photoperiods of Tatsuno and Sagamihara strains.
Fig. 3. Photoperiod-dependent diapause of 2 geographic strains (Tatsuno and Sagamihara) of *B. dorsalis* at 24 °C. The closed circles are the observations of Tatsuno strain; the solid lines are the values of Tatsuno strain estimated by loess; the open circles are the observations of Sagamihara strain; the dashed lines are the values of Sagamihara strain estimated by loess; the closed square is the 50% diapause incidence at the critical photoperiod of Tatsuno strain (11.74 h); the open square is the 50% diapause incidence at the critical photoperiod of Sagamihara strain (12.07 h).

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REFERENCES CITED

ANKERSMIT, G. W., AND ADKISSON, P. L. 1967. Photoperiodic responses of certain geographical strains of *Pectinophora gossypiella* (Lepidoptera). J. Insect Physiol. 13: 553-564.

BONATO, O., LURETTE, A., VIDAL, C., AND FARGUES, J. 2007. Modelling temperature-dependent bionomics of *Bemisia tabaci* (Q-biotype). Physiol. Entomol. 32: 50-55.

BRIERE, J. F., PRACROS, P., LE ROUX, A. Y., AND PIERRE, J. S. 1999. A novel rate model of temperature-dependent development for arthropods. Environ. Entomol. 28: 22-29.

CAMPBELL, A., FRAZER, B. D., GILBERT, N., GUTIERREZ, A. P., AND MACKAUER, M. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11: 431-438.

CLEVELAND, W. S. 1979. Robust locally weighted regression and smoothing scatterplots. J. Amer. Statist. Assoc. 74: 829-836.

CLEVELAND, W. S., GROSSE, E., AND SHYU, W. M. 1991. Local regression models, pp. 309-376. In J. M. Chambers and T. J. Hastie [eds], Statistical models in S. Chapman & Hall, London.

DAVISON, A. C., AND HINKLEY, D. V. 1997. Bootstrap methods and their application. Cambridge University Press, Cambridge.

DAVISON, A. C., AND KUONEN, D. 2002. An introduction to the bootstrap with applications in R. Statistical Computing & Statistical Graphics Newsletter 13(1): 6-11.

EFRON, B, AND TIBSHIRANI, R. 1993. An introduction to the bootstrap. Chapman & Hall, New York, London.

ELIOPOULOS, P. A., KONTODIMAS, D. C., AND STATHAS, G. J. 2010. Temperature-dependent development of *Chilocus bipustulatus* (Coleoptera: Coccinellidae). Environ. Entomol. 39: 1352-1358.

GOMI, T. 1997. Geographic variation in critical photoperiod for diapause induction and its temperature dependence in *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). Oecologia 111: 160-165.

GOTOH T., SUGIMOTO, N., PALLINI, A., KNAPP, M., HERNANDEZ-SUAREZ, E., FERRAGUT F., HO, C. C., MI-GEON, A., NAVAJAS, M., AND NACHMAN, G. 2010. Reproductive performance of seven strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. Exp. Appl. Acarol. 52: 239-259.

HONG, J. W., AND PLATT, A. P. 1975. Critical photoperiod and daylength threshold differences between northern and southern populations of the butterfly *Limenitis archippus*. J. Insect Physiol. 21: 1159-1165.

IKEMOTO, T. 2005. Intrinsic optimum temperature for development of insects and mites. Environ. Entomol. 34: 1377-1387.

IKEMOTO, T. 2008. Tropical malaria does not mean hot environments. J. Med. Entomol. 45: 963-969.

IKEMOTO, T., AND TAKAI, K. 2000. A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. Environ. Entomol. 29: 671-682.

ISHIHARA, M., AND SHIMADA, M. 1999. Geographical variation in photoperiodic response for diapause induction between univoltine and multivoltine populations of *Kytorhinus sharpianus* (Coleoptera: Bruchidae). Environ. Entomol. 28: 195-200.

ITO, K., AND NAKATA, T. 2000. Geographical variation of photoperiodic response in the females of a predatory bug, *Ortis sauteri* (Poppius) (Heteroptera: Anthocoridae) from northern Japan. Appl. Entomol. Zool. 35: 101-105.

JAROSIK, V., HONEK, A., AND DIXON, A. F. G. 2002. Developmental rate isomorphy in insects and mites. Am. Nat. 160: 497-510.

JAROŠIK, V., KRATOCHVIL, L., HONEK, A., AND DIXON, A. F. G. 2004. A general rule for the dependence of developmental rate on temperature in ectothermic animals. Proc. R. Soc. Lond. B (Suppl.) 271: S219-S221.

KROON, A., VEENENDAAL, R. L., AND VEERMAN, A. 1997. Photoperiodic induction of diapause in the spider...
mite *Tetranychus urticae*: qualitative or quantitative time measurement? Physiol. Entomol. 22: 357-364.

Kuang, X., Parajulee, M. N., Shi, P., Ge, F., and Xue, F. 2011. Testing the rate isomorphy hypothesis using five statistical methods. Insect Sci. (in press, doi 10.1111/j.1744-7917.2011.01428.x).

Kurota, H., and Shimada, M. 2003. Photoperiod-dependent adult diapause within a geographical cline in the multivoltine bruchid *Bruchidius dorsalis*. Entomol. Exp. Appl. 106: 177-185.

Lactin, D. J., Holliday, N. J., Johnson, D. L., and Craig, R. 1995. Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24: 68-75.

Logan, J. A., Wollkind, D. J., Hoyt, S. C., and Tani-goshi, L. K. 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5: 1133-1140.

Miller, W. E. 2011. Temperature-dependent development in capital-breeding Lepidoptera. J. Lepid. Soc. (in preparation).

Van Rijn, P. C. J., Mollema, C., and Steenhuis-Broers, G. M. 1995. Comparative life history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bull. Entomol. Res. 85: 285-297.

Schoolfield, R. M., Sharpe, P. J. H., and Magnuson, C. E. 1981. Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. J. Theor. Biol. 88: 719-731.

Sharpe, P. J. H., and Demichele, D. W. 1977. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64: 649-670.

Shi, P., Ge, F., and Men, X. 2010. How to compare the lower developmental thresholds. Environ. Entomol. 39: 2033-2038.

Shi, P., Ikemoto, T., Egami, C., Sun, Y., and Ge, F. 2011. A modified program for estimating the parameters of the SSI model. Environ. Entomol. 40: 462-469.

Stacey, D. A., and Fellowes, M. D. E. 2002. Temperature and the development rates of thrips: evidence for a constraint on local adaptation. Eur. J. Entomol. 99: 399-404.

Sun, L., He, H., and Xue, F. 2007. Geographic variation of diapause in insects. Acta Agri. Univ. Jiangxi. 29: 922-927 (in Chinese).

Taubner, M. J., and Tauber, C. A. 1972. Geographic variation in critical photoperiod and in diapause intensity of *Chrysopa carnea* (Neuroptera). J. Insect Physiol. 18: 25-29.

Taubner, C. A., Tauber, M. J., and Nechols, J. R. 1987. Thermal requirements for development in *Chrysopa Oculata*: a geographically stable trait. Ecology 68: 1479-1487.

Taylor, F. 1981. Ecology and evolution of physiological time in insects. Am. Nat. 117: 1-23.

Timer, J., Tobin, P. C., and Saunders, M. C. 2010. Geographic variation in diapause induction: the grape berry moth (Lepidoptera: Tortricidae). Environ. Entomol. 39: 1751-1755.

Wang, R., Lan, Z., and Ding, Y. 1982. Studies on mathematical models of the relationship between insect development and temperature. Acta Ecol. Sin. 2: 47-57 (in Chinese).

Xue, Y., and Chen, L. 2007. Statistical Models and R Software. Tsinghua University Press, Beijing. pp. 255-258 (in Chinese).