

Cumulative meta-analysis: a new tool for detection of temporal trends and publication bias in ecology

Roosa Leimu^{*} and Julia Koricheva

Section of Ecology, Department of Biology, University of Turku, FIN-20014 Turku, Finland

Temporal changes in the magnitude of research findings have recently been recognized as a general phenomenon in ecology, and have been attributed to the delayed publication of non-significant results and disconfirming evidence. Here we introduce a method of cumulative meta-analysis which allows detection of both temporal trends and publication bias in the ecological literature. To illustrate the application of the method, we used two datasets from recently conducted meta-analyses of studies testing two plant defence theories. Our results revealed three phases in the evolution of the treatment effects. Early studies strongly supported the hypothesis tested, but the magnitude of the effect decreased considerably in later studies. In the latest studies, a trend towards an increase in effect size was observed. In one of the datasets, a cumulative meta-analysis revealed publication bias against studies reporting disconfirming evidence; such studies were published in journals with a lower impact factor compared to studies with results supporting the hypothesis tested. Correlation analysis revealed neither temporal trends nor evidence of publication bias in the datasets analysed. We thus suggest that cumulative meta-analysis should be used as a visual aid to detect temporal trends and publication bias in research findings in ecology in addition to the correlative approach.

Keywords: cumulative meta-analysis; publication bias; plant–herbivore interaction; paradigm shift

1. INTRODUCTION

Significant changes in the magnitude and even the direction of research findings with time have recently been reported in several fields of ecology (Alatalo et al. 1997; Simmons et al. 1999; Poulin 2000; Nykänen & Koricheva 2004). Initially, these temporal trends have been considered as isolated occurrences and attributed to paradigm shifts, scientific fads, changes in methodological approaches or biases in the choice of study systems. However, a recent analysis of 44 independent datasets, covering a wide range of ecological and evolutionary topics (Jennions & Møller 2002), revealed that temporal changes in the magnitude of effect sizes represent a general phenomenon in ecology; thus a more general explanation for the occurrence of these changes must be sought. It has been suggested that the most likely reason for the observed decreases in the strength of findings over time is underreporting or delayed publication of non-significant results (Jennions & Møller 2002). However, while publication bias against non-significant results has been observed in medicine (Song et al. 2000), no direct evidence of such bias has been found in ecology (Møller & Jennions 2001; Koricheva 2003). Another type of publication bias which may explain the decrease in the strength of research findings with time is the underreporting or delayed publication of results which contradict the predictions of the prevailing theories (Alatalo et al. 1997; Jennions & Møller 2002). To the best of our knowledge, however, no convincing evidence of this kind of bias has been found in ecology so far. In the absence of an adequate explanation, the decline observed in the strength of ecological findings with time raises questions

about the validity and applicability of any general conclusions drawn from the ecological literature.

We believe that considerable progress in understanding the patterns and causes of changes in the magnitude of reported effects with time could be achieved by using a more efficient method for detecting and analysing these changes. Temporal trends in the strength of research findings in ecology have traditionally been studied by estimating correlations between the magnitude of the effect size and publication year (Poulin 2000; Jennions & Møller 2002). This method however, is applicable only when the magnitude of the effect exhibits a uniform and monotonous decrease or increase with time, which is not always the case (Nykänen & Koricheva 2004). Here, we introduce another method, known as cumulative meta-analysis, which provides a framework for following the evolution of treatment effects over time as evidence accumulates. It is commonly used in the medical sciences, but is new in ecological and evolutionary research (but see Nykänen & Koricheva 2004). Cumulative meta-analysis is a series of metaanalyses in which studies are added to the analysis based on a predetermined order, and changes in the magnitude of the mean effect and its variance are followed (e.g. Chalmers 1991; Lau et al. 1995). Studies can be arranged, for example, by year of publication, by the size of the study, by the size of the difference between treatment and control groups, or by any other covariate (Lau et al. 1995; Rosenberg et al. 2000). Unlike correlation analysis, cumulative meta-analysis reveals uneven irregular changes in effect size as well as multiple shifts in opposite directions. Moreover, cumulative meta-analysis can also be used to detect possible publication bias.

Here, we illustrate the use of cumulative meta-analysis in ecology by examining temporal trends in two independent *Author for correspondence (roosa.leimu@utu.fi). Sets of studies testing plant defence theories (Koricheva et al.

1998; Koricheva 2002). We also use cumulative metaanalysis to reveal a possible publication bias against studies reporting disconfirming evidence, by examining whether such studies are published in lower quality journals compared with studies which report results supporting the prevailing theory.

2. METHODS

The first dataset used in this study is from the meta-analysis of studies testing the carbon–nutrient balance (CNB) hypothesis (Koricheva et al. 1998). The CNB hypothesis postulates that the balance between carbon and nutrient availability determines the levels of secondary metabolites in plants (Bryant et al. 1983). According to the CNB hypothesis, concentrations of carbonbased secondary compounds (CBSCs) such as phenolics will increase in plants if growth is reduced more than photosynthesis, or if photosynthesis is enhanced more than growth. The production of CBSCs will, on the contrary, be decreased by factors that reduce photosynthesis more than growth or stimulate growth more than photosynthesis. This hypothesis has commonly been tested by subjecting plants to fertilization, shading and elevated carbon dioxide. Because there are differences in the magnitude and direction of changes in CBSCs in response to the above treatments, we have selected from among the papers reviewed in Koricheva et al. (1998) only those that measured changes in concentrations of CBSCs in response to nitrogen fertilization, as these studies were the most abundant. The measure of the effect size in this dataset is Hedges' d, calculated as the difference between the mean CBSC concentrations in fertilized and control groups, divided by the pooled standard deviation and weighted by a correction term that eliminates small-sample bias (Koricheva et al. 1998; Rosenberg et al. 2000). A negative effect size indicates a reduction in CBSC concentrations in response to fertilization, a result consistent with the predictions of the CNB hypothesis.

The second dataset is from the meta-analysis on costs of plant antiherbivore defences (Koricheva 2002). Plant defence theories predict that antiherbivore defences will be costly, which may result in a trade-off between resource allocation to defence, or to growth and reproduction (Herms & Mattson 1992). The magnitude of defence costs is usually evaluated by measuring phenotypic or genetic correlations between defence and fitness measures. Genetic correlations are measured less frequently and tend to be higher than phenotypic correlations (Koricheva 2002). We have therefore included in the analysis only phenotypic correlations between defence and fitness. The measure of effect size in this data set is the z-transformed Pearson's correlation coefficient between plant defence and fitness measures (Rosenberg et al. 2000). A significant negative correlation is considered to be evidence of a defence cost.

It has been predicted that temporal trends and publication bias are more likely to occur in hypothesis-driven research (Poulin 2000). We have therefore selected from the two datasets, only those studies that were aimed specifically at testing the CNB hypothesis or the cost-of-defence hypothesis (as stated in the introductions of the original papers); more descriptive studies and studies testing other hypotheses were excluded. When several estimates of effect size were available per study (e.g. for different types of CBSCs or defence measures), we pooled the data by study and calculated an overall effect for each study. Our final datasets each consisted of 31 studies published during 1984–1999 (tests of the CNB hypothesis) and 1975–1999 (tests of the cost-of-defence hypothesis).

Cumulative meta-analyses were conducted using the META-W_{IN}, v. 2.0 statistical program (Rosenberg et al. 2000). In the first analysis, the data in both datasets were sorted in chronological order. The earliest available study was entered into the analysis first. At each step of the cumulative meta-analysis, one more study was added to the analysis and the mean effect size and 95% confidence interval (CI) were recalculated (Rosenberg et al. 2000). The formulas for calculation of cumulative mean effect sizes and their CIs are the same as in the traditional meta-analysis (e.g. Gurevitch et al. 2001); the only difference is that the mean is not calculated for the whole group of studies at once but is instead recalculated each time a new study is added to the analysis. This allows estimation of the contribution of individual studies, and the evolution of the magnitude and direction of research findings can be followed in more detail. Studies published in the same year were entered into the analysis in random order. To control for the fact that several studies were usually published in the same year, we also conducted separate cumulative meta-analyses in which the data were pooled by year, i.e. a single effect size was calculated for each year. This approach may be preferred if the total number of studies and the time-span of meta-analysis are large, and studies are very unevenly distributed among years.

Another type of cumulative analysis was conducted to exemplify the use of cumulative meta-analysis to reveal publication bias. We sorted studies according to the impact factor of the journal in which the study was published and examined changes in the magnitude and direction of the effect with an increase in the journal impact factor. The impact factors of journals were obtained from the Journal Citation Reports of the Institute for Scientific Information (ISI). Studies published in the same journal were entered into the analysis in random order.

The mean effect size was considered significant if its 95% CI did not include zero. The data were analysed using a fixed effects model because the estimate of the pooled variance was less than or equal to zero (Rosenberg et al. 2000). Typically, as studies are added to the analysis the cumulative effect size first changes greatly from one analysis to the next, but gradually stabilizes around the mean value for the whole set of studies. However, when the magnitude of the effect size depends on the variable examined (in our case publication year or journal impact factor), no stabilization of the cumulative effect size takes place. We also examined the relationship between effect size and publication year or journal impact factor by calculating Pearson correlation coefficients, in order to compare the results of the traditional correlative approach and cumulative meta-analysis.

3. RESULTS AND DISCUSSION

Correlation analyses revealed no significant association between the magnitude of effect size and publication year in either of the two datasets (CNB: $r = 0.0186$, $p = 0.921$, $n = 31$; defence cost: $r = 0.164$, $p = 0.377$, $n = 31$; [fig](#page-2-0)ures $1a$ and $2a$). By contrast, the cumulative meta-analysis revealed clear temporal changes in the magnitude of reported effects in both datasets (figures $1b$, c and $2b$, c). In the CNB data, a few early studies conducted in 1984–1987 reported significant reductions in CBSCs concentrations in response to nitrogen fertilization, as predicted by the CNB hypothesis. However, as the diversity of plant species tested and chemical compounds measured increased, the evidence for the CNB hypothesis became weaker; by the late 1980s and early 1990s, the magnitude of the CBSC response to nitrogen fertilization had become

Figure 1. Temporal changes in reported responses of woody plants to nitrogen fertilization in terms of concentrations of CBSCs. Effect size is calculated as the difference between the mean CBSC concentration in fertilized and control groups, divided by the pooled standard deviation and weighted by a correction term that eliminates small-sample bias. Error bars represent 95% CI. Effect size is considered statistically significant if its 95% CI does not overlap zero (marked by vertical lines). Negative effect size indicates a reduction in CBSC concentrations in response to fertilization, the result consistent with the predictions of the CNB hypothesis. Horizontal dashed lines in (a) and (b) denote the first observation from each publication year (marked on (c)). (a) Effect sizes and 95% CI of 31 individual studies published in 1984–1999, in chronological order. Cumulative effect sizes and 95% CI for the same 31 studies calculated by (b) study and (c) year. Analysis begins with the chronologically oldest study (at the bottom of the graph); at each step the effect size from the next study in chronological order (b) or the mean effect for the next year (c) are added to the analysis, and mean effect sizes and 95% CI are recalculated. In (b) , effect sizes from studies published in the same year are added into the analysis in random order.

non-significant (figure $1b,c$). It became evident that the CNB hypothesis does not satisfactorily predict the responses of slow-growing tree species adapted to a resource-limited environment (Bryant et al. 1987) and does not apply to many types of CBSCs, such as metabolites with a rapid turnover (Reichardt et al. 1991) and terpenoids (Björkman et al. 1991; Muzika 1993). The scope of the CNB hypothesis was thus restricted, and more recent studies have mainly examined the responses of phenylpropanoids. As a result, in the cumulative meta-analysis based on individual studies, the magnitude of the effect increased and once again became significant in the late 1990s, while the variance around the mean effect fell considerably (figure 1b). When the data were pooled by year, variance was also reduced by the late 1990s, but the increase in the magnitude of the effect size was less evident (figure $1c$).

The results from the dataset on the costs of antiherbivore defences revealed similar temporal changes (figure $2b,c$). Early studies conducted in 1975–1990 demonstrated significant negative correlations between defence and fitness measures, supporting the hypothesis of defence costs. However, in the early 1990s, the magnitude of reported costs decreased and became non-significant ([figure 2](#page-3-0)b,c). This may be because of the fact that several theoretical studies published in the late 1980s provided theoretical justification for the absence of costs. For instance, it was demonstrated that positive correlations among life-history characteristics are to be expected when the variation in resource allocation is small (Van Noordwijk & de Jong 1986) or when trade-offs involve more than two traits (Pease & Bull 1988). More recent studies have thus been designed to enable the detection of trade-offs under the conditions where they are most likely to occur. This could result in a slow but steady increase in the magnitude of the effect size since 1996 in the analysis based on individual studies [\(figure 2](#page-3-0)b). This latter pattern, however, was not clearly seen when the data were pooled by year [\(figure 2](#page-3-0)c).

Previous investigations in other fields of ecology have found that the strength of findings usually decreases over time (e.g. Simmons et al. 1999; Poulin 2000; Jennions & Møller 2002; but see Alatalo et al. 1997). Cumulative analysis revealed a similar pattern in the two datasets analysed. In the CNB dataset, the magnitude of the reported effect decreased from -1.01 in 1984 to -0.26 (analysis based on individual studies) and -0.36 (analysis based on years) in 1999. In the cost-of-defence dataset, the magnitude of the effect size decreased from -0.33 in 1975, to -0.17 (in the analysis based on individual studies) and -0.10 (in the analysis based on years) in 1999. This decrease, however, was uneven, and in both datasets a shift towards an increase in effects was found at the end of the time-period reported (figures $1b$ and $2b$). Similar but more drastic temporal changes have been found in studies testing the effects of previous damage on woody plant chemistry and insect herbivore performance (Nykänen & Koricheva 2004).

Figure 2. Temporal changes in reported magnitude of cost of antiherbivore defences. Effect size is a z-transformed Pearson's correlation coefficient between plant defence and fitness. Error bars represent 95% CI. Effect size is considered statistically significant if its 95% CI does not overlap zero (marked by vertical lines). Significant negative effect sizes are considered as evidence of defence cost. Horizontal dashed lines in (a) and (b) denote the first observation from each publication year (marked on (c)). (a) Effect sizes and 95% CI of 31 individual studies published in 1975–1999, in chronological order. Cumulative effect sizes and 95% CI for the same 31 studies calculated by (b) study and (c) year. Analysis begins with the chronologically oldest study (at the bottom of the graph); at each step the effect size from the next study in chronological order (b) or the mean effect for the next year (c) are added to the analysis, and mean effect sizes and 95% CI are recalculated. In (b) , effect sizes from studies published in the same year are added into the analysis in random order.

Figure 3. Cumulative meta-analysis plot for reported changes in CBSCs in response to nitrogen fertilization as a function of the impact factor of the journal in which the study was published. Analysis is based on the same 31 studies as in [figure 1](#page-2-0); the measure of the effect size is Hedges' d (see [figure 1](#page-2-0)), error bars represent 95% CI. The impact factor is marked next to the first study published in this journal included in the analysis. Analysis begins with the study published in the journal with the lowest impact factor (at the bottom of the graph); at each step the effect size from the next study in the order or increasing impact factor is added to the analysis, and mean effect sizes and 95% CI are recalculated. Effect sizes from studies published in the same journal are added into the analysis in random order.

On the basis of the temporal trends revealed in the present study and in Nykänen & Koricheva (2004), we identified three stages in the development of the evidence for the ecological hypothesis: (i) supportive evidence in early tests of a newly formulated hypothesis; (ii) the accumulation of disconfirming evidence, often accompanied by the formulation of alternative scenarios and competing theories; and (iii) reformulation of the original hypothesis, leading to restriction of its scope and gradual stabilization of effect size. These three stages basically correspond to the three stages of theory development: early, immature and mature (Loehle 1987; Stamp 2003). It is important to realize that meta-analyses of studies on the same topic conducted during different stages of the process of evidence accumulation may produce different results. The magnitude of the effect size is likely to be considerably overestimated in the first stage and somewhat underestimated in the second one. Only in the third stage, when the magnitude of the effect stabilizes and the variance around the mean decreases, does the mean effect size adequately describe the magnitude of the effect.

Although clear, the temporal changes detected in the magnitude of effects in the two datasets were gradual, supporting the prediction of Paine (2002) that advances in ecological understanding are usually achieved not through paradigm shift (sensu Kuhn 1970), but by conceptual evolution. The fact that we found no paradigm shifts is not very surprising, given the relatively short time-span of the datasets, and—more importantly—the nature of ecology and evolutionary research as scientific disciplines. Although temporal changes have been reported in these fields (e.g. Alatalo et al. 1997; Simmons et al. 1999; Jennions & Møller 2002; Nykänen & Koricheva 2004), hardly any studies have found clear paradigm shifts or revolutions in the sense of Thomas Kuhn (Paine 2002; but see Nykänen & Koricheva 2004). This has been explained by the fact that ecology is not a linear discipline; progression is often uneven through time, as interest in particular theories and hypotheses waxes and wanes (Paine 2002). Because of this non-linearity of ecology, cumulative meta-analysis can be especially useful in this discipline.

Two recent studies in ecology have reported associations between the impact factor of the journal in which the paper is published and the absolute magnitude of the reported effect size (Murtaugh 2002) or the proportion of nonsignificant results in a study (Koricheva 2003). A positive association was found between journal quality and the strength of an effect (Murtaugh 2002), whereas the proportion of non-significant results tended to be negatively associated with journal impact factor (Koricheva 2003). We therefore conducted a cumulative meta-analysis according to the impact factor of the journal, in order to determine whether place of publication is influenced by the sign and magnitude of the reported effect. For the CNB dataset, correlation analysis revealed no significant association between the magnitude of the effect size reported in a study and the impact factor of the journal in which the study was published $(r = -0.078, p = 0.68, n = 31)$. By contrast, cumulative meta-analysis revealed striking differences in the magnitude and sign of the effects published in journals with low and high impact factors [\(figure 3\)](#page-3-0). Studies published in journals with an impact factor of less than unity reported results contrasting with the predictions of the CNB hypothesis. However, when studies published in journals with a higher impact factor were successively added to the analysis, the magnitude of the treatment effect began to decrease and became non-significant. Finally, when studies published in journals with impact factors of more than three were added to the analysis, the sign of the effect changed, indicating a significant reduction in CBSC concentrations in response to nitrogen fertilization, as predicted by the CNB hypothesis. In other words, studies supporting the CNB hypothesis tend to be published in higher-quality journals than studies contradicting it. To our knowledge, this is the first demonstration that the publication fate of ecological studies may be affected not only by the absolute magnitude and statistical significance of the reported effect, but also by its sign. We have tested whether the observed relationship between the direction of the effect and the impact factor of the journal was a result of the tendency of earlier studies (which provided stronger support for the CNB hypothesis) to be published in journals with a higher impact factor than later studies. This was not the case; there was no correlation between the impact factor of the journals in which individual papers were published and the publication year ($r = 0.072$, $p = 0.700$, $n = 31$). The observed pattern may instead be a result of a tendency of authors to submit studies with findings contradicting the prevailing theory or hypothesis to lower quality journals, to a tendency of the referees and editors of major journals to reject such studies, or to both. However, it is possible that publishing decisions may be influenced by the quality of the study, e.g. experimental design or methodology, rather than by whether or not the results support the hypothesis. The quality of a study, however, should not influence the direction of the findings in such a way as to cause the observed pattern between the magnitude and sign of an effect and journal quality. In the dataset on costs of defence, no clear relationship was found between magnitude and direction of effect size and journal impact factor (not shown).

We have shown that cumulative analysis can be used both for the examination of temporal trends and for the detection of publication bias in ecological studies. The advantage of using cumulative meta-analysis compared with the traditional correlative approach is supported by the results of the correlation analyses, where no significant relationships were found between magnitude of effect size and publication year or journal impact factor. The fact that the results of the cumulative meta-analysis differed from those of the correlation analysis can be explained by the uneven and irregular pattern of the temporal changes, which is not revealed by the correlative approach. Cumulative meta-analysis is also more informative than correlation analysis because it examines the behaviour over time of the variation around the mean effect, thus indicating whether the direction and magnitude of an effect have stabilized over time and allowing meaningful assessment of the mean effect size. We thus recommend that cumulative meta-analysis should be used in addition to the traditional correlative approach when examining temporal changes and trends in research findings in ecology.

Meta-analysis is nowadays widely used in ecology as a powerful way to summarize the results of independent studies on a given topic (Gurevitch et al. 2001). It is important to realize, however, that accumulation of scientific evidence is a dynamic process, which cannot be satisfactorily described by the mean effect size calculated at a single time-point. The temporal changes in the magnitude and even the direction of effects reported in the present and several earlier ecological studies indicate that meta-analyses of studies on the same topic conducted at different points in time may lead to different conclusions. Updating the results of meta-analysis on a given topic at regular intervals by including newly published studies is a standard practice in medicine (Chalmers & Haynes 1994), which should also be adopted in ecology. Cumulative analysis represents a useful tool for updating summary results as evidence accumulates; it should be applied in a number of other ecological fields, to find out whether the three phases identified in the development of plant defence theories, and the publication bias against studies reporting disconfirming evidence, represent a general phenomenon.

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