The Meaning of Percentage Parasitism Revisited: Solutions to the Problem of Accurately Estimating Total Losses from Parasitism

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ABSTRACT
An earlier forum article identified a need for new analytical methods for obtaining stage-specific estimates of losses from parasitism for life table, population dynamics, and evaluation studies. A discussion of the conceptual framework for recently developed solutions to this problem is presented. Classes of solutions considered include recruitment, stage frequency, and death rate analyses. We present the rationale and methodology for each and compare their relative usefulness for systems of varying types of biologies and sampling constraints. These solutions provide tools for the more accurate quantification of parasitism as a mortality factor in insect life systems.

KEY WORDS Insecta, parasitism, biological control, death rates

STUDIES AIMED AT EVALUATING the effect that insect parasitoids have on their host populations require methods to quantify accurately the proportion of hosts that enter the stage susceptible to parasitism and the number that subsequently become parasitized. Commonly used methods have focused on levels of parasitism in field samples. Such data typically have been reported either as seasonal trends of percentage parasitism, peak values of sample percentage parasitism, or a pooled value formed by combining all samples in a season. These approaches rarely provide an effective measure of total losses to parasitism for a stage over a generation (Van Driesche 1983).

In the last 6 yr, the analytical bases of several new methods to estimate total losses from parasitism have been developed and applied to field populations (e.g., Van Driesche & Bellows 1988; Bellows et al. 1989a,b; Gould et al. 1989; Hazzard et al. in press; Lopez & Van Driesche 1989; Van Driesche et al. 1989, in press; Gould 1990; Gould et al. 1990, in press). In this paper, we review these methods as parts of an overall framework for estimating losses to parasitism. We consider three categories of solutions—measurement of recruitment, stage-frequency analysis, and death rate analysis—and compare their strengths and weaknesses for estimating total losses from parasitism.

The Problem: A Recapitulation
Before describing the developments that constitute a solution to the problem articulated in Van Driesche (1983), we present a brief restatement of the problem and the reason for its significance to quantifying the effect of natural enemies in biological control and populational ecology.

Because the population of an insect stage typically begins to lose members through death or development to the next stage in the life cycle before the entire recruitment to the stage is completed, at no time are all members of the generation present to be counted. This idea is analogous to a sink partly filled with water (i.e., the population), into which water is flowing (recruitment) and from which water is draining (death or advancement to the next stage) (shown schematically in Fig. 1a). To construct a life table, we need to know the total numbers that enter a stage (in this analogy, the total amount of water entering the sink). What biologists typically measure, however, is the number of animals present per sample unit at points in time (which is analogous to the amount of water in the sink at any given time). Although it is true that the volume of water present at any time is determined by the moment-to-moment balance of cumulative influx minus cumulative outflow, if these latter quantities are not known, it is not possible to determine total inflow from even the most detailed set of observations on the quantity of water in the tank at fixed moments in time. What is needed is a continuous record of recruitment for the whole period over which animals enter the stage of interest for the generation. This can be achieved by measuring recruitment for a series of contiguous intervals spanning the whole period when recruitment occurs (e.g., Van Driesche & Bellows 1988). When the goal is to assess not only how many insects enter a given life stage over the course of a generation, but also to determine how many of that number subsequently become parasitized, the problem is compounded because the basic problem discussed above now applies to two quantities that
must be measured; i.e., the total number of hosts recruited and the number that subsequently become parasitized. The linkages between these values are both dynamic and complex (Fig. 1b).

Although there are some systems in which biology and life history characteristics are such as to produce nondynamic systems not subject to these problems (for example, cases where the sampled stage is a diapause stage and accumulates without loss as, for example, is approximately the case for gypsy moth eggs, because dead or parasitized eggs remain countable) or systems such as some leaf-miners in which lost insects continue to be traceable in samples through their remains, the majority of insects do have overlapping recruitment and losses. For these cases, densities and percentage parasitism values seen in samples do not measure adequately the level of parasitoid effect.

**Some Solutions**

**Recruitment Analysis.** Where technically feasible, shifting from the measurement of densities per sample unit at moments in time (i.e., beetle eggs per square meter or larvae per plant on individual dates), to numbers of the stage recruited per sample unit per unit of time is an elegant and powerful solution to the problems raised in Van Driesche (1983). In terms of the hydraulic model analogy, this amounts to replacing the depth gauge in the tank with a flow meter in the intake pipe. When an unbroken series of recruitment (or input) measurements are made for a series of back-to-back intervals spanning the entire period over which new hosts arise, total host recruitment to the stage is simply the sum of the recruitment values for all intervals. If, in an exactly parallel manner, gains are measured to the category of "parasitized hosts" (this being equivalent to parasitoid recruitment except for the case of gregarious parasitoids), the ratio of total parasitoid recruitment to total host recruitment is then an unbiased estimate of total losses in the stage due to parasitism. This approach, which we term "the recruitment method," has been applied both to species with discrete generations (e.g., *Pieris rapae* (L.) larvae parasitized by *Cotesia glomerata* L. in Massachusetts [Van Driesche & Bellows 1988]) and to continuously breeding species (e.g., the cabbage aphid, *Brevicoryne brassicae* (L.), parasitized by *Diaeretiella rapae* McIntosh [Lopez & Van Driesche 1989]). It is important to note that, because recruitment rates will change over time, measurements must be made continuously over the entire period of interest. Single or scattered estimates of recruitment for short periods will not allow estimation of total recruitment for the generation.

Conceptually, the method is extremely simple. One measures directly the quantities desired rather than trying to infer or calculate them from host density and data on percentage parasitism in samples, which are generally complex and difficult to decipher. The feasibility of the method hinges on being able to design sample regimens that in fact measure host and parasitoid recruitment to the stages of interest, given the biologies of the insects involved and the features of the host plant or habitat in which they occur.
Several methods have been used to measure recruitment to the host population. Birley (1977), for example, reported recruitment of Aeneolamia varia saccharina (Distant) through the use of emergence traps which recorded insects per square meter per time period as the insects left the soil to join the above-ground population. Van Driesche & Bellows (1988) measured recruitment of *P. rapae* to its first instar via a "double sampling" scheme in which sets of randomly selected collard plants were stripped of all *P. rapae* larvae on one day and reexamined 3-4 d later, counting all first or second instars present. Because there is no movement of these very small larvae between plants, such larvae found on previously stripped plants are recruits that have entered the population by the hatching of eggs present at the first examination or ones laid shortly afterward. (It should be noted that missed larvae not found on the first examination will inflate recruitment estimates, whereas larvae missed on the second examination will lower estimates. These biases should be quantified to estimate the accuracy of the recruitment estimates obtained.) Hazzard et al. (in press) measured recruitment to the egg stage of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), through a process of periodically examining a fixed cohort of sample plants (every 3-4 d) and using color-coded tags to record permanently the dates of the interval when each egg mass was first observed. Van Driesche et al. (in press), in a separate study, measured recruitment of Colorado potato eggs using the same double sampling scheme as previously applied to *P. rapae* larvae. Lopez & Van Driesche (1989) estimated recruitment of the cabbage aphid, *B. brassicae*, to its first nymphal instar by cross-multiplying adult aphid densities found in field samples by the birth rate of randomly selected females from the field population held in leaf cages for successive short time intervals. This approach required that three categories of adult aphids (healthy, parasitized, and diseased) be tracked separately as each differed from the others in its per-capita rate of offspring production. (Such recruitment estimates based on reproduction of females in leaf cages may be somewhat higher than the actual population values because these adults are of necessity protected from disturbance by other aphids, predators, and parasites while in leaf cages.) Undoubtedly, as the concept of measuring recruitment to a stage is applied to more species, an ever-expanding set of procedures to make such measurements will be developed.

Two approaches have been developed to measure parasitoid recruitment (that is, the rate of gain to the category of parasitized hosts): the "short marker stage method" and the "trap host method."

The short marker stage method is illustrated for *C. glomerata* by Van Driesche & Bellows (1988) and Van Driesche (1988). It consists of developing a recognizable "picture" of a sufficiently early parasitoid stage (e.g., egg or first instar) such that, in view of the development rate of the parasitoid and the heat accumulation in the field between sample dates, all hosts in a sample that are found upon dissection to bear this parasitoid stage can be considered to have become parasitized in the interval between samples. For *C. glomerata*, for example, parasitoid egg length was found to be a very sensitive measure of egg age. The simple process of recording the number of parasitized hosts in dissection samples that bore parasitoid eggs of less than a critical length was sufficient to allow parasitoid recruitment to be scored. Recruitment may be measured by collecting samples at intervals of sufficiently short duration that parasitoids cannot both enter and develop past the marker stage in one interval and summing this recruitment for successive intervals during the generation.

Lopez & Van Driesche (1989) measured recruitment of *D. rapae* in the same manner, except that hosts were incubated for 48 h at 25°C before dissection to cause parasitoid eggs to advance to the first instar because eggs were too small to be seen in dissection. The precise details of development rate, intersample length, and postsampling procedures thus determine what stage is the critical one for signifying parasitoid recruitment.

The second approach to measuring parasitoid recruitment, termed the trap host method, involves placing nonparasitized hosts (usually from laboratory or field insectary colonies) in the field for short intervals, after which they are recovered. The proportion parasitized is then determined by rearing or dissection. This proportion is then multiplied by the average density of unparasitized hosts during each sample interval in the field population. This approach has been applied to *C. glomerata* (Van Driesche 1988, Van Driesche & Bellows 1988), *D. rapae* (Lopez & Van Driesche 1989), *Cotesia melanoscela* (Ratzenburg) and *Parastigma silvestris* (Robineau-Desvoidy) (Gould 1990), *Brachymeria intermedia* (Nees) (Gould et al. in press), and *Edouven puttleri* (Van Driesche et al. in press). The performance of this method has been variable. For *P. rapae* larvae, it performed as well as the short marker stage method (Van Driesche 1988). Similarly, for sessile host stages, the performance of the method has generally been good (e.g., Colorado potato beetle eggs [Van Driesche et al. in press] and gypsy moth pupae [Gould et al. in press]). However, for mobile hosts, host behaviors can create problems if trap hosts fail to act "naturally." For example, Gould (1990) suggests that in the gypsy moth system, trap host larvae failed to exhibit the same diel pattern of migration between trunk and canopy, remaining permanently in the canopy and thus experiencing less parasitism than was likely to have occurred in the wild population (Gould 1990). Furthermore, for systems where several larval or nymphal instars are attacked by the parasitoid with varying degrees of success or preference, it may be impossible to deploy all instars as trap hosts, and attack rates on
only a single instar may poorly reflect attack rates for the aggregate population of all susceptible hosts. For example, cabbage aphid nymphs born to adults in 3–4-d intervals were used by Lopez & Van Driesche (1989) as trap hosts. These were largely second instars when exposed, a preferred instar (Hafez 1961). Although attack rates on these trap hosts probably reflected that of first to third instars reasonably well, attack rates on fourth instars and adults were lower and thus not well predicted from attack rates on the second instars used as trap hosts.

In addition to providing direct estimates of total losses to parasitism, measurement of host and parasitoid recruitment adds power to the interpretation of trends seen in density data. Recruitment and density data taken together can be used to estimate interval–specific survival rates of healthy and parasitized hosts as separate categories (Van Driesche & Bellows 1988). This allows exploration of whether or not these classes of hosts suffer different rates of mortality in the field from factors other than parasitism. Differential mortality, when it occurs, strongly influences resulting levels of sample percentage parasitism; thus, it is important to be able to recognize its occurrence. Also, when both density and recruitment to a single stage are measured, the overall survival rate of that stage can be estimated without any need for data on the next stage in the animal’s life history (the usual method). For example, Van Driesche et al. (1989) developed such an approach by modifying the stage–frequency analysis technique of Richards & Waloff (1954) to estimate losses from predation suffered by Colorado potato beetle eggs. For cases where a parasitoid is known to be the sole or major source of mortality, this method may be used to estimate total losses from parasitism in the stage.

In summary, wherever sampling procedures can be developed to measure host and parasitoid recruitment directly, we believe this should be done because the resultant estimates of percentage parasitism are superior to those produced by any other known method of analysis. Cases do exist, however, where this is not possible. In the following section, we consider how total losses to parasitism in a stage for a generation can sometimes be made when only densities and sample percentage parasitism can be measured or when these can be measured together with either host or parasitoid recruitment, but not both.

Analysis of Stage–Frequency Data and Percentage Parasitism. The problem of deriving estimates of numbers entering successive stages in an insect’s life history using data consisting solely of densities of one or more stages has been an area of long-standing interest, and many methods have been proposed (e.g., Richards & Waloff 1954; Dempster 1956; Richards et al. 1960; Southwood & Jepson 1962; Kiritani & Nakanoji 1967; Manly 1974, 1976, 1977, 1989; Ruesink 1975; Bellows & Birley 1981; Bellows et al. 1982). Each method imposes certain limitations in terms of assumptions and data requirements. The problem, however, of simultaneously estimating, through some type of stage frequency analysis procedure, the numbers entering two interacting stages (i.e., the host stage susceptible to parasitism and the “immature stage” of the parasitoid) has been addressed very little and is considerably more complex than the single-species case.

Simmonds (1948) recognized how differing developmental times of healthy hosts versus immature parasitoids could distort parasitism rates seen in samples and proposed a formula intended to correct for this. Miller (1954) also recognized the problem and suggested that samples for measuring levels of parasitism be timed to occur after parasitoid oviposition was complete but before parasitoid emergence had begun. Smith (1965), following upon ideas first outlined by Thompson (1955), proposed a method of computing a “seasonal index of parasitism” from samples spaced far enough apart so that parasitoids in parasitized hosts present at the time of one sample have all emerged by the time of the next sample. These early efforts to identify and correct for the problem of obtaining accurate estimates of mortality from parasitism using density and sample percentage parasitism data failed to alter fundamentally how estimates were made, and “trend data” (i.e., percentage parasitism versus date for the study) and pooled estimates of parasitism (all samples combined into a single sample) continued to be the approaches commonly used.

More recently, several workers have addressed these same issues and have proposed solutions very similar to those advocated earlier. Russell (1987) proposed a method of using the ratios of developmental times of the immature parasitoid (the stages associated with the parasitized host) and that of the corresponding stage of the healthy host to “correct” estimates of parasitism seen in field samples. This approach is very similar to that of Simmonds (1948). Alternatively, Hill (1988) advocates estimating percentage parasitism by Apanteles ruflcns (Holiday) of larvae of the noctuid Mythima separata (Walker) by limiting collections to only the middle (fourth or fifth) instars which are sufficiently young so that parasitoid emergence has not yet started, an approach reminiscent of that advocated by Miller (1954). Neither of these approaches provides a comprehensive analytical framework within which the strength of the method can be evaluated. They remain intuitive adjustments of uncertain value.

Although in principle, any of the methods of estimating numbers entering a stage for one species could be extended to the case of doing so simultaneously for stages of two interacting species, the method of Southwood & Jepson (1962), being graphical rather than computational, has attracted the most attention. Kolodny-Hirsch et al. (1988) applied the method to a study of C. melanoscelsa parasitism of gypsy moth, Lymantria dispar (L.),
larvae, and Schneider et al. (1988) did so to measure the effect of *Tetrastichus ceroplastae* (Girault) on the Florida wax scale, *Ceroplastes floridensis* Comstock. Neither of these studies, however, analyzed the actual causes, but only the effect of parasitism they used. In contrast, Bellows et al. (1989b) presented a complete theoretical analysis of the biases inherent in seven variations of the Southwood & Jepson (1962) method extended to the two-species case. The conclusions of this work reconfirmed that relationships between density and percentage parasitism data are extremely complex and that it is very difficult to obtain accurate estimates of total losses to a stage from such data. Specifically, the analysis showed the Southwood process to be subject to potentially large biases arising from three sources: (1) the rate of parasitism, (2) rates of mortality of parasitized hosts from causes other than parasitism, and (3) rates of mortality of healthy hosts from causes other than parasitism. The paper by Bellows (1989b) analyzes how varying rates of each of these factors combine to affect estimates of parasitism and specifies under what conditions the overall bias in each variation is low enough for accurate use. The paper points out that uncorrected application of the Southwood & Jepson process would be disastrous, with estimates of percentage parasitism in error by hundreds of percent. However, given knowledge of the biologies of the insects under study and mortality rates occurring in the population, applications of the method are appropriate in some special cases, primarily when deaths from parasitism and other factors total less than 20%. Even the simplest cases, however, were subject to significant biases.

Other methods of stage–frequency analysis are potentially adaptable to this problem, but to date, none has been so extended. Experience with the Southwood & Jepson (1962) technique suggests that biases involved are likely to be large and complex. Although there are likely to be specific circumstances where use of such methods may be appropriate, uncorrected development and use of intuitive ad hoc extensions of such methods outside of a fundamental analytical understanding of the biases inherent in the analysis should be avoided.

**Death Rate Analysis.** A third class of systems for which estimates of total parasitism are sometimes needed are those in which neither densities nor recruitment rates can be measured, but only mortality rates. Certain insects, particularly forest insects, borers, and insects that live in soil, pose sampling difficulties of such magnitude that neither recruitment rates nor stage densities can be readily measured. In the absence of these types of data, the question arises if stage losses from a mortality factor can be estimated solely from death rates seen in samples. A method to do so for mortality agents attacking the gypsy moth has been developed (J. S. E. [unpublished data]; Gould et al. 1990). The method consists of collecting individuals at frequent intervals, rearing them under field temperature conditions, and noting the proportion that die from each cause in the interval from one sample to the next. Algebraic equations given by Gould et al. (1990) and Buonaccorsi & Elkinton (1990), when applied to the observed numbers dying in these samples, provide estimates of the original percentage of the sample that was parasitized. The aggregate loss in the stage or population to a factor may be calculated from the losses to that factor for each interval. In its simplest form, this method requires that all hosts have entered the susceptible stage before the first sample and that no host recruitment occurs during the sampling period. The method extends the ideas of Royama (1981) and is capable of separating the contributions of two or more mortality factors (e.g., two parasitoids, etc.) that act together contemporaneously within a single sampling interval.

**Conclusions**

Estimating the proportions of a host population attacked by a parasitoid remains an important objective, both in evaluation of natural enemies and in the proper construction of life tables. A traditional approach to approximating this proportion by collecting samples of individuals and simply determining the proportion of individuals parasitized in samples has long been recognized to be of extremely limited value (Van Driesche 1983).

Three broad classes of analytical approaches have undergone recent development specifically for determining percentage losses to parasitism: stage–frequency analysis, direct measurement of recruitment, and death rate analysis. A widely used method of stage frequency analysis, the graphical approach of Southwood & Jepson (1962), has been extended to the case of interacting host and parasitoid populations. Except under limited circumstances, the method is subject to large and complex biases (Bellows et al. 1989b). Other techniques in this class, although potentially useful, have not yet been developed analytically for this purpose. Once this has occurred, applications also are likely to be limited to specific cases by significant biases as was true of the Southwood graphical method. Consequently, continued collection of only host density and percentage parasitism data (without concomitant recruitment data) are not, in our opinion, to be recommended. Tools to derive unambiguous estimates of stage losses to parasitism from such data do not exist at this time except in special cases.

The direct measurement of recruitment resolves many of the problems associated with both sample percentage parasitism and stage–frequency analysis. This method is conceptually simple and appears robust to both analytical and sampling assumptions. The recruitment method also is potentially applicable to the study of stage losses to pathogens, providing early infections are detectable either visibly in host dissections or by some...
laboratory test such as the use of ELISA-type antigen-antibody tests (e.g., McGuire & Henry 1989) or DNA hybridization (e.g., Keating et al. 1989).

Death rate analysis (J.S.E., unpublished data; Gould et al. 1990) provides a useful approach for systems lacking prolonged host recruitment and for which density or recruitment estimates cannot be made readily. It is capable of separating the effects of several factors acting together on a stage and for summing the effect of factors whose impact is spread over several life stages.

These three classes of analytical procedures provide solutions for estimating percentage parasitism in many situations. They have already proved useful in both discretely breeding and continuously breeding populations and in situations where recruitment can be measured and where it cannot. The action of contemporaneous factors can be separately quantified by proper application of some of these techniques (Buonaccorsi & Elkinton 1990, Gould et al. 1990). The development of additional methods will expand the scope of situations in which proper analytical procedures will be available for estimating percentage parasitism.

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