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On estimating the duration of phenological stages in bryophytes

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Estimating the duration of stages in the life cycle of bryophytes based on repeated censuses of populations is a non-trivial task. A method to estimate the length of the period of primary moult in birds is shown here to be also applicable for mosses, using the stage between the events of loss of the calyptra and operculum loss as an example. The duration of this 'operculum' stage varied considerably among nine Dutch moss species studied. The species formed three clusters, with average duration of this stage of 8, 23 and 101 days.

Sexual reproduction of bryophytes is a complex, tightly coordinated process involving a sequence of phenological stages, from the production of gametangia and gametes, via the fertilisation of eggs to the development and maturation of the sporophytes. The whole process, including the transitions between the various stages, is usually strongly seasonal, as documented repeatedly since more than a century (Arnell 1875, Grimme 1902, Van der Wijk 1960). The length of the reproductive cycle as a whole and the duration of the individual stages may differ considerably between species, however, even within the same region, depending on their life histories and ecology (Stark 2002). Comparisons between populations and species have been made easier by a standardized subdivision of the life cycle. The subdivision proposed by Greene (1960), somewhat adjusted by Forman (1965), Longton and Greene (1969) and others (Table 1), is nowadays in common use.

Detailed studies of the phenology of particular species show, that there is also quite some variation among individuals within populations in this respect (Hancock and Brassard 1974). As a consequence, it is often difficult to estimate, with accuracy, how long each individual stays in each stage, and how much variation there is among species and individuals within species in the duration of particular stages. An added complication is that it may be difficult to monitor individual shoots for long periods, especially in the case of dense cushions or turfs of small shoots.

A method to obtain such data from repeated censuses has been described for the time that birds spend in primary moult by Underhill and Zucchini (1986). In this paper I show, that their method is also applicable to bryophytes, and I use it to estimate for nine species the duration of one particular stage, that of the sporophyte without calyptra but with the operculum still intact. This stage roughly corresponds to the EOI and LOI stages in Table 1 (although sporophytes in the EOI stage may still have their calyptra). It was chosen as the target stage, because in this way each sporophyte could relatively easily and unambiguously be assigned in the field to one of the three stages involved (sporophyte with calyptra, sporophyte without calyptra but with operculum intact and sporophyte with operculum fallen).

Material and methods

From 1990 to 2003 phenological stages were monitored on sporophytes in populations of nine moss species, mostly in the dunes near Amsterdam (4°32'E, 52°20'N), but occasionally in other parts of the Netherlands (see Table 2 for periods of observation for each species). Within delimited areas I included all populations with more than ca 20 sporophytes in the analysis. During the period of sporophyte maturation the populations were censused on a weekly basis; at each census the number of sporophytes in the stages sporophyte with calyptra, sporophyte without calyptra but with operculum intact and sporophyte with operculum fallen were counted, if necessary with help of a 10× magnifying glass. For brevity the stages will be des-

Table 1. Stages of sporophytic development in mosses (after Greene 1960 and Miles et al. 1989).

Abbreviation	Stage	Event marking beginning of stage
SV	swollen venter	venter of archegonium begins to swell
ECP	early calyptra in perichaetium	calyptra becomes recognizable and begins to emerge from perichaetial bracts
LCP	late calyptra in perichaetium	calyptra becomes half exserted from bracts
ECI	early calyptra intact	calyptra becomes fully exserted from bracts
LCI	late calyptra intact	swelling of capsule begins
EOI	early operculum intact	operculum becomes brown in colour
LOI	late operculum intact	capsule becomes brown in colour
OF	operculum fallen	operculum falls
EF	empty and fresh	>75% of spores are shed
A	aborted	apex of sporophyte withers prior to spore formation

ignated as the calyptra stage, the operculum stage and the operculum lost stage from now on.

Data analysis

At first sight, it would seem that the length of the period during which the sporophytes are in the operculum stage can be derived from a linear regression of the numbers of sporophytes without calyptra against time. As Underhill and Zucchini (1988) and Underhill (1993) pointed out, this method and other similar methods based on simple linear regression cannot be used, because the variance of the data at the beginning and at the end of the observation period (when the fraction of sporophytes which lost their calyptra is close to 0 and close to 1, respectively) is much smaller than in the middle of the period. This results in an incorrect estimate of the length of the stage of interest. Underhill and Zucchini (1988) presented a better method, originally used for the statistical description of avian primary moult and based on a different underlying statistical model, and their method has been applied here to calculate the duration τ of the operculum stage. Because the model calculations are not easy, an outline of the calculation, completed with an example, is given. This outline has mainly been extracted from Underhill and Zucchini (1988); further details concerning methodology and formulas can be found in their paper.

The random variable T is the number of days from some reference date at which a randomly selected sporophyte loses its calyptra and so makes the transition from the calyptra stage to the operculum stage. Let $F_T(t)$ be the probability distribution function and $f_T(t)$ the probability density function of T. In this paper $F_T(t)$ is taken to be normally distributed with mean μ and standard deviation σ, although other distributional forms of practical interest can be used here such as Laplace, Exponential or Gamma. It is supposed that the duration of the operculum stage, τ, is the same for all individual sporophytes.

Apart from the developmental duration, τ, for the sporophytes in the operculum stage, the main quantities of interest are the expectation, μ, and the standard deviation, σ, of T, which give the mean starting date and spread of starting dates of the operculum stage in the population. However, once estimates of $\hat{F}_T(t)$ and $\hat{\tau}$ are available, they can be used to estimate any relevant population characteristic. For example, the proportion of the population which has completed the operculum stage by day t_0 can be estimated using $\hat{F}_T(t_0 - \hat{\tau})$. The proportion of the population in the operculum stage on day t_0 is estimated using $\hat{F}_T(t_0) - \hat{F}_T(t_0 - \hat{\tau})$.

The data consists of observations of random samples of sporophytes in the three stages at roughly weekly intervals. Let Y(t) be the fraction of sporophytes without a calyptra, but with the operculum still intact, at time t selected at random from the population. Note that Y(t) is a random variable whose probability distribution function, $F_{Y(t)}(y)$, changes with time t.

The objective is to estimate τ and $F_T(t)$ from observations on the process Y(t). This situation is somewhat unusual in that we wish to estimate the distribution of a random variable, T, for which no direct observations are available.

The following relationship provides the basis for achieving this:

$Y(t) \le y$ if and only if $T > t - y\tau$, $0 \le y \le 1$ (1)

from which it follows that

$F_{Y(t)}(y) = 1 - F_T(t - y\tau)$, $0 \le y \le 1$

Suppose that the sample under consideration consists of I sporophytes having a calyptra, J having an operculum, and K not having an operculum anymore. Let the days on which these observations were made be $t_1, t_2, \ldots t_I$; $u_1, u_2, \ldots u_J$; $v_1, v_2 \ldots v_K$, respectively. We denote these vectors of observation days by t, u and v, respectively.

Table 2. Years of data collection on capsule phenology per species.

Brachythecium albicans (Hedw.) Schimp.	1991–1992
	1992–1993
Brachythecium rutabulum (Hedw.) Schimp.	1990–1991
	1991–1992
	1992–1993
	1994–1995
	1997–1998
	2001–2002
Hypnum cupressiforme Hedw.	1991–1992
	1992–1993
	1994–1995
Hypnum jutlandicum Holmen & E.Warncke	2001–2002
Orthotrichum anomalum Hedw.	1997
Rhynchostegium megapolitanum (F.Weber & D.Mohr) Schimp.	1991–1992
Rhynchostegium murale (Hedw.) Schimp.	1991–1992
Syntrichia ruralis (Hedw.) F.Weber & D.Mohr var. *arenicola* (Braithw.) J.J.Amann	1992
	1993
	1996
Tortula subulata Hedw.	1993
	1994

The likelyhood of these observations under the model is

$$L(\theta,t,u,v) = \prod_{i=1}^{I} P(t_i) \quad \prod_{j=1}^{J} Q(u_j) \quad \prod_{k=1}^{K} R(v_k)$$

where $\theta = (\tau,\mu,\sigma)$ is the vector of parameters, and where

$P(t) = \Pr\{Y(t) = 0\} = 1 - F_T(t)$

$Q(t) = \Pr\{0 \le Y(t) \le 1\} = F_T(t) - F_T(t-\tau)$

$R(t) = \Pr\{Y(t) = 1\} = F_T(t-\tau)$

The maximum likelihood estimators of the parameters are those values of τ, μ and σ which maximize (Eq. 1), or, equivalently, its logarithm. This gives finally a set of three equations in the three unknowns τ, μ and σ. In regression terms, these equations can be seen as the three normal equations. We need for the solutions the first and second partial derivatives of the log likelihood function, L. Finally we have the following set of equations in matrix notation:

$$\begin{pmatrix} H\tau\tau & H\tau\mu & H\tau\sigma \\ H\mu\tau & H\mu\mu & H\mu\sigma \\ H\sigma\tau & H\sigma\mu & H\sigma\sigma \end{pmatrix} \begin{pmatrix} \tau \\ \mu \\ \sigma \end{pmatrix} = \begin{pmatrix} h\tau \\ h\mu \\ h\sigma \end{pmatrix}$$

where the capital H's are the second partial derivatives of the log likelihood function, L with respect to the the parameters (τ,μ,σ) and the small h's are the first partial derivatives of the log likelihood function, L with respect to the the parameters (τ,μ,σ).

For this model it is not possible to find an analytical solution for these 'normal' equations, and numerical methods have to be used instead. We use here a Newton-Raphson iteration to solve the normal equations. The end situation is, that if the iteration converges, we have found values for τ, μ and σ.

Results

I first present the data of *Brachythecium rutabulum* for the year 1994–1995 as an example of the type of results that can be obtained using this complex set of operations. The capsule data collected in this case are shown in Table 3. After running the program, the following solutions were found for this example:

duration of the operculum stage – τ = 58.1 [days]

mean starting day – μ = 160.1 [days] (day 1 is 1 June 1994)

standard deviation of starting day – σ = 15.8 [days]

Fig. 1 visualizes the comparison of observed to fitted values.

Altogether, the dataset for *B. rutabulum* spanned a period of five years (Table 2). The results (Table 4) demonstrate, that there is considerable year-to-year variation in all three parameters estimated: the date at which the first calyptra was shed varied from day 149 (28 October) to

Table 3. Field data for *Brachythecium rutabulum* in the years 1994–1995.

Day no.	Date	Calyptra	Operculum	Open	Calyptra %	Operculum %	Open %
144	23 Oct 1994	556	63	0	89.8223	10.1777	0
150	29 Oct 1994	1092	570	0	65.704	34.296	0
157	5 Nov 1994	360	553	0	39.4304	60.5696	0
164	12 Nov 1994	322	1270	0	20.2261	79.7739	0
172	20 Nov 1994	264	650	0	28.884	71.116	0
178	26 Nov 1994	108	670	0	13.8817	86.1183	0
184	2 Dec 1994	213	1360	0	13.541	86.459	0
191	9 Dec 1994	150	965	0	13.4529	86.5471	0
198	16 Dec 1994	62	1300	30	4.45402	93.3908	2.15517
206	24 Dec 1994	30	1300	50	2.17391	94.2029	3.62319
212	30 Dec 1994	17	1100	250	1.2436	80.4682	18.2882
228	15 Jan 1995	5	70	1690	0.283286	3.96601	95.7507
234	21 Jan 1995	5	27	1736	0.282805	1.52715	98.19
241	28 Jan 1995	0	1	200	0	0.497512	99.5025

day 187 (5 December), and the mean operculum stage lasted from 55 to 97 days.

As the number of years of observation for the other species was much smaller, I present here only the mean results over all observation years for the species (Table 5). The results clearly show large differences between the species, which may be classified into three clusters based on the length of the operculum stage (Fig. 2). Cluster 1 includes the two species of *Brachythecium* and those of *Hypnum*, and is characterized by very long operculum stages (86–120 days). Cluster 2 contains the two *Rhynchostegium* species and *Tortula subulata*, and includes the species with moderately long operculum stages (18–30 days). In the two species of cluster 3, *O. anomalum* and

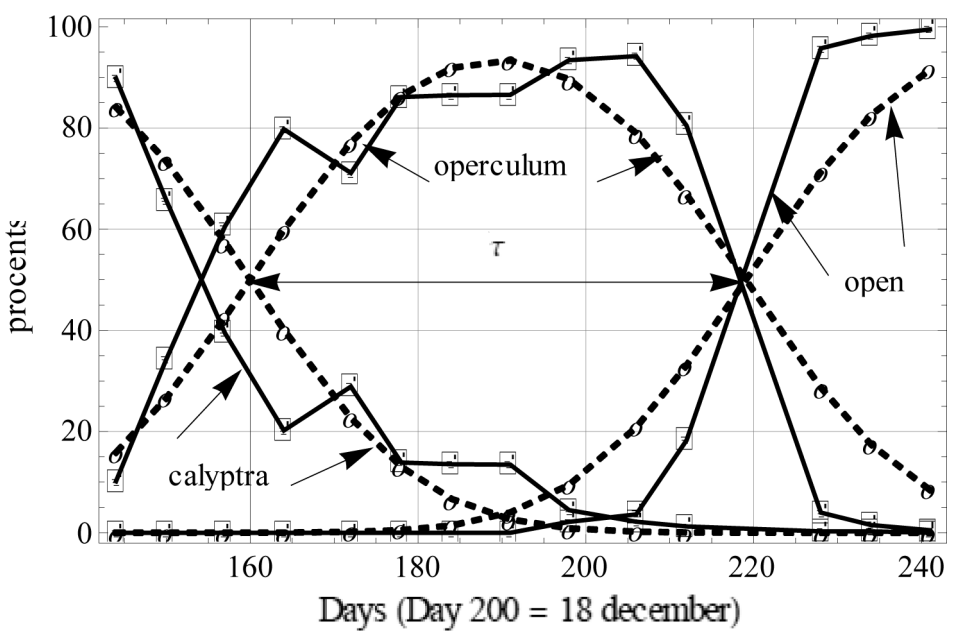

Figure 1. Comparison of observed (crosses, solid lines) to fitted (circles, dashed lines) data for the proportion of capsules with calyptra, without calyptra but with operculum still intact and without operculum, for *Brachythecium rutabulum* in the years 1994–1995.

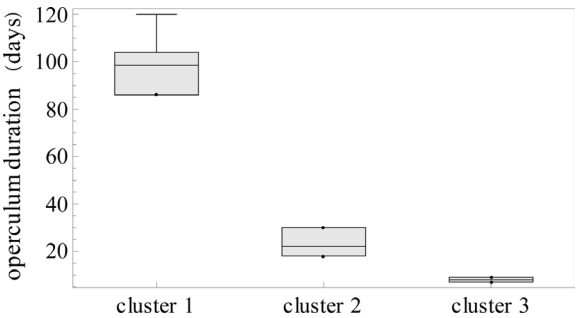

Figure 2. Boxplot of the three clusters of Dutch moss species (Table 5) on the basis of the operculum duration τ.

Table 4. Estimates of the duration of the operculum stage (τ, the mean starting date μ (days since 1 June) and its standard deviation σ, for *Brachythecium rutabulum*.

Year	τ [days]	μ [days]	σ [days]
1991–1992	70	187	27
1992–1993	97	149	28
1994–1995	58	160	16
1997–1998	55	174	16
2001–2002	84	151	19

S. ruralis var. *arenicola*, the operculum stage lasts only for 7–8 days.

As the duration of the operculum stage may also depend on the speed of development of the sporophyte as a whole, I have also recorded the period during which the sporophyte is clearly visible on the plant. As shown in Fig. 3, the nine target species differ considerably in the length of this period, from 2.5 months for *O. anomalum* to almost nine months for *H. cupressiforme*.

Discussion

While the physical presence of a calyptra has been shown to be very important for the 'normal' development of the sporophyte (Bopp 1956, 1957, French and Paolillo Jr. 1975, Budke et al. 2011), much less is known about the importance of the presence of an operculum in the final stage of sporophyte development. One can speculate, that the intact operculum protects the inside of the capsule and the spore mass against fast desiccation and attacks of micro-organisms and small animals. Whether this would also provide the conditions for 'normal' development of the peristome, for example, is quite likely but as far as I am aware of not yet demonstrated.

The length of the operculum stage as defined in this paper is determined by the moment the calyptra is shed on the one hand, and the shedding of the operculum itself on the other hand. Differences between the three clusters of species may be due to various factors. One might assume, that in some species the presence of the calyptra remains important for the completion of the development of the sporophyte anatomy almost until the stage of spore release, while in other species the final stages of development may not be so critically dependent on the presence of the calyptra. On the other hand, it may simply be that in species with long-rostrate opercula the calyptra does not fall off early because of physical reasons. This might explain the difference between *Brachythecium* spp. (with conical opercula) and *Rhynchostegium* spp. (with rostrate opercula), and the very long time that the calyptra is staying on the long-rostrate opercula of *S. ruralis* and *T. subulata*, but does not provide an explanation for the situation in *O. anomalum*. Future experiments on the influence of the presence of a calyptra on the final stages of sporophyte development may shed some light on this topic.

Another simple explanation of the differences between the three clusters might be sought in differences in the length of the period of total sporophyte development – long operculum stage duration might be caused by slow development of the sporophyte in general. In view of my own data as well as the data of previous authors on these species, this explanation does not seem very plausible. There is a weak relation between duration of the opercu-

	mrt	apr	mei	jun	jul	aug	sep	okt	nov	dec	jan	feb	mrt	apr
Bra.alb	----	----	----	----	----	----	-xxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxx-
Bra.rut	----	----	----	----	----	-xxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxx-
Hyp.cup	----	----	----	----	-xxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	----
Hyp.jut	----	----	----	----	----	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	x---
Ort.ano	---x	xxxx	xxxx	x---	----	----	----	----	----	----	----	----	----	----
Rhy.meg	----	----	----	----	----	----	-xxx	xxxx	xxxx	xxxx	xxxx	xxxx	----	----
Rhy.mur	----	----	----	----	----	----	----	---x	xxxx	xxxx	xxxx	xxxx	xxxx	x---
Syn.rur	xxxx	xxxx	xxxx	xxx-	----	----	----	----	----	----	----	----	----	----
Tor.sub	----	xxxx	xxxx	xxxx	xxxx	xxx-	----	----	----	----	----	----	----	----

Figure 3. Capsule period duration during the year. Start determined by presence of clearly visible sporophytes with discernable calyptra; the period ended when the last capsules were empty. Four xxxx-signs represent one month.

Table 5. Estimates of the duration of the operculum stage (τ, the mean starting date μ (days since 1 June), its standard deviation σ and cumulative total number of capsules, n, counted in the field for nine moss species.

Cluster	Species	τ [days]	μ [days]	σ [days]	n [-]
1	Bra.alb	104	158	23	52042
1	Bra.rut	93	151	31	97804
1.	Hyp.cup	86	123	32	46603
1	Hyp.jut	120	123	23	7001
3	Ort.ano	7	118	11	695
2	Rhy.meg	18	179	29	2688
2	Rhy.mur	22	215	26	968
3.	Syn.rur	9	111	17	30293
2	Tor.sub	30	151	26	1284

lum stage and the period of visible sporophyte presence in the field (Fig. 3), but a comparison with the developmental period from fertilisation to spore release does not produce any pattern. For example, the sporophytes of *B. rutabulum* develop relatively fast (9–12 months from fertilization to spore release, Lackner 1939), but have about the longest duration of the operculum stage, but in *S. ruralis* sporophyte development takes 14–16 months, while the operculum stage lasts for only nine days. Perhaps, the presence of a calyptra lasts longer in species with more complex peristomes, but I have not found any data to support this assumption.

The results clearly show, that the method of Underhill and Zucchini (1986) is suitable in a wider range of studies on the duration of developmental phases than only the duration of primary moult in birds. In fact, it allows more accurate estimates of the duration of any stage that is characterized by two observable, fast-occurring events and is constrained seasonally, without having to monitor individual organisms through the time period involved. With regard to bryophytes, it might be used to improve our knowledge of the developmental timing of antheridia and archegonia by frequently revisiting (preferably large) populations, at each occasion collecting a number of randomly chosen stems and checking these for the developmental stage of archegonia and/or antheridia. As the starting event one might take the first observation of recognizable immature archegonia c.q. antheridia, and as the closing event the maturity of an archegonium c.q. the rupture of the first antheridium. This may help to clarify which bryophytes tend to be proandrous or protogynous, a topic of considerable interest from a life-history point of view (De Jong et al. 2008). Similarly, it might be worthwhile to study the time between meiosis (usually recognizable by the turning red of the annulus of the sporophyte) and the moment of spore release. One might expect, that the rate of such developmental processes may depend on temperature and moisture conditions and so, on latitude, oceanicity and habitat. For such larger-scale comparisons, more data will be needed, however.

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