

Further experimental investigations on variation of types, and on the nature of quantitative differences in Daphnia types.

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Abstract

The following is a translation from the German of Richard Woltereck's classic paper¹ introducing the idea of reaction norms; a concept which encapsulated the now-ubiquitous idea of a genotype-by-environment interaction, and contributed to the idea of the developmental program. Woltereck begins with a brief discussion of Lamarckist and Selectionist views on heritability, the influence of the environment ("milieu"), and variation of types. This is followed by a discussion of methods for understanding these concepts in biological systems. A research plan is outlined for distinguishing between these possibilities in a fashion to be generally convincing among the diverse factions. Data following the initial steps of this research plan are presented. An introduction to the model system of Daphnia is provided, and in particular the interesting response of head morphology to seasonal variation ("cyclomorphosis"). The basic observation of consistent responses to the environment among clonal Daphnia is presented and analyzed in light of both temperature and nutritional variation, and in terms of physiology and morphogenesis. [TO BE CONTINUED]

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Outline

Preface: General. – Necessary Materials. – Isolation of the Elementary Types. – Plan of Investigation. – Observations on the Crossing and Selection of Daphnia.

1. Ascertainment and Analysis of the Quantitative character "head-height".
 - (a) The basis of different head-heights.
 - (b) Assessment and description of the collected phenotypes.
 - (c) Genotypic basis of head-height and different genotypes.

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¹The text is drawn from pages 110-173 of the *Verhandlungen der Deutschen Zoologischen Gesellschaft*, vol. 19, 1909, as digitized by the Biodiversity Heritage Library (<http://www.biodiversitylibrary.org/item/182001>). I copyrighted the translation under my own name for lack of a better idea.

Preface

From the results of many experiments, in particular botanical studies of inheritance of the last century—on the one hand the Mendel's crosses, on the other the inbreeding experiments of Johannsen, and most lately the famous mutations of de Vries—we have derived a new concept in the existence of the variation of types. This concept, which is championed especially by de Vries and W. Johannsen², stands in nearly-irreconcilable contrast to the Lamarckist inclinations of today's Botany, and also of most zoologists, regarding whether they consider themselves Lamarckists or Selectionists.

We hear the following: the elementary types (not concerning ourselves with the artificial umbrella term of systematic "species") differ from each other through the assortment of predispositions, determinants, or genes, which are inherited in their germplasm. So far the "exact doctrine of heredity" (Johannsen) corresponds to the older teachings, especially Weismann. Moreover, this doctrine provides the most brilliant evidence for the existence and continuity of the germplasm-elements that Weismann could wish for, with regard to the correctness of his theoretical ideas. The dissent of the new teachings concerns only the variation inherent in these elements:

1. These hereditary elements are of fixed quantities, which do not show the smallest variability in siblings (respective in germplasm to their parents), or show any gradual variation across many generations. A change in the elements, and thereby the heritable characters of the elementary type, follows exclusively through spontaneous mutations; fluctuating, individual, or continuous variability is negligible with respect to the heritable nature of types.
2. The nature of change of the hereditary elements, and therefore the direction of these mutations, does not have

²Compare especially Johannsen's new book: *Elemente der exakten Erblichkeitslehre*. Jena, G. Fischer, 1909 (515 S.).

See also the report of Arnold Lang in this journal. Finally I would like to draw attention to the important work of the Heidelberg botanist Georg Klebs, without which I could not have completed the present work. Klebs has demonstrated the exceptional scope of continuous, milieu-dependent variability in several plants (*Sempervivum*, *Sedum*). See also his "Studien ueber Variation" (*Archiv f. Entwicklungsmech.* 1907)

a causal relationship to the local conditions, or milieu. Extreme changes of milieu may prefer the emergence of certain mutations, and thereby “disturb the equilibrium of the germplasm”, but the thus-unleashed variation of germplasm and type does not indicate any causal relationship of the mutation to the milieu.

The milieu in no way affirms the direct inheritance of variation of type (Lamarckism), and even its indirect action (by harvesting the most adequate variants; that is, Selectionism) is secondary. Selection does not decree the multitudes of always-available, always-fluctuating germ-variants (and absolutely cannot impart a direction on the organs, tissues, or germ cells); its scope is restricted to the occasionally-appearing final mutations. Here it will eradicate the unworkable mutants through the preference for certain forms, at least so far as they have not already left the stage through inviability or sterility.

Therefore: there is no continuously-arising variation and equally no continuously-arising evolution.

And: there is no determining influence of the milieu, except through the excision of unfavorable mutants.

These are hard blows against that conception of the development of the organic world in which we are tutored. However, the zoologists present so many illuminating examples of finely-graduated arrays of forms and adaptations that it is difficult to accept a conception of discontinuous development, and that by these little steps of type-variation the influence of the milieu (whether direct or indirect) can be explained away, without giving up our lone mental anchor to an unmetaphysical understanding of organic development.

However, the advocates of the “exact doctrine of heredity” support themselves with analytical experiments, and therefore they see all the rich body of facts that have accumulated since Darwin as inconsequential—dashing our hopes, like a tanner who loses his hides [*translator's note: ????]*. Of course: experimental analysis is that rich goal towards which observational facts are only the smallest steps.

Therefore, if we want to meaningfully fight against the new doctrines we must use the weapons of the enemy, above all analytical experiments. Then we will compare the worth the “old” conception: continuous variation of type under a determining milieu; to the “new”: discontinuous variation without a determining milieu. Then it will be clear that the latter conception, of mutations, is proven experimentally.

Necessary Materials for Investigating the Variation of Types.

If we wish to assess precisely whether organic properties vary continuously or in jumps, it would be best to make use of predefined quantitative characters (e.g. length measurements). In these characters, variation can be numerically and unambiguously specified. It is my opinion a weakness in the new doctrine that Johannsen presents, that he considers only qualitative categorical characters (e.g. color) that are altogether subjective. We must take greater pains to eliminate the influence of crossing and sex in our experiments, such that only sexless, parthenogenetic, or self-fertilizing organisms are appropriate. In this sense, it is indispensable that we

study variation and heredity in “clean lines” (Johannsen).

Third, finally, it is preferable that we only have to do with organisms that are very diverse in form [trans.: alternatively, numerous], in order that small (but heritable) differences can be discerned. The comparative study will then concern itself with forms that show no substantial difference, other than one or a few quantifiable contrasts, which in comparison constitute elementary types (“Biotypes” for Johannsen).

The familiar Danish botanist succeeded in cleanly identifying such elementary types in great numbers out of landraces of grains and beans. We require similarly numerous and diverse animals for zoological studies to design a comparable experiment, for instance Daphnia or another Cladocerans that are available in our lakes and ponds. For an account of landraces in Daphnia, I can recommend my own work showing different heritable Biotypes³.

Both of the other requirements are fulfilled either in Johannsen's work, or in my Daphnia. Johannsen isolated “clean lines” through self-fertilization, whereas I used parthenogenetic propagation under controlled, constant culture conditions while removing the eggs with ephippia (egg casings generated during sexual reproduction), such that the fertile eggs develop into ♀♀. Thus the correctly-timed isolation and robust feeding of these ♀♀ suffices across many Daphnia races to maintain clean lines (in some cases for this purpose, the sibling ♀♀ without ephippia should be excluded). Almost invariably under favorable conditions, the animals revert from laying more ephippia to making parthenogenetic eggs (subitan eggs), and indeed almost without exception they are ♀♀ individuals that arise out of such (“post-ephippial”) virgin eggs. The appearance of ♂♂ in unattended cultures can be dismissed, so long as no ephippia appear; to this end, new generations were obtained by descent through single ♀♀ individuals, to entirely exclude this possibility.

As is well known, Johannsen used as quantitative features the length of beans and the serration of barley varieties. In Daphnia, the length of the carapace spines, the height of the head, the facial angle, the spine angle (compare figures 1 and 2), and sexuality (see below) can serve as quantitative characters.

Thus are the materials which Johannsen used and my own Daphnia populations sufficiently comparable, at least in these three critical points, in spite of the totally unrelated morphologies of higher plants and metazoans.

Isolation of the Elementary Types

As is well known, Johannsen proceeded from the premise that he isolated variants comprising different elementary types or Biotypes that were mixed in an initial, apparently uniform population. These types proved themselves through the heritability of coherent, unchanging features. The progeny of each individual, for instance the largest and smallest, show similar means and the same range of variation as their parents. (Johannsen's rightly-thrilling results were, of course, that selection on the variants of an elementary type does not reveal any influence of heritability on vari-

³Verh. d. D. Zool. Ges. 1908, vol. 18, pp. 234-240

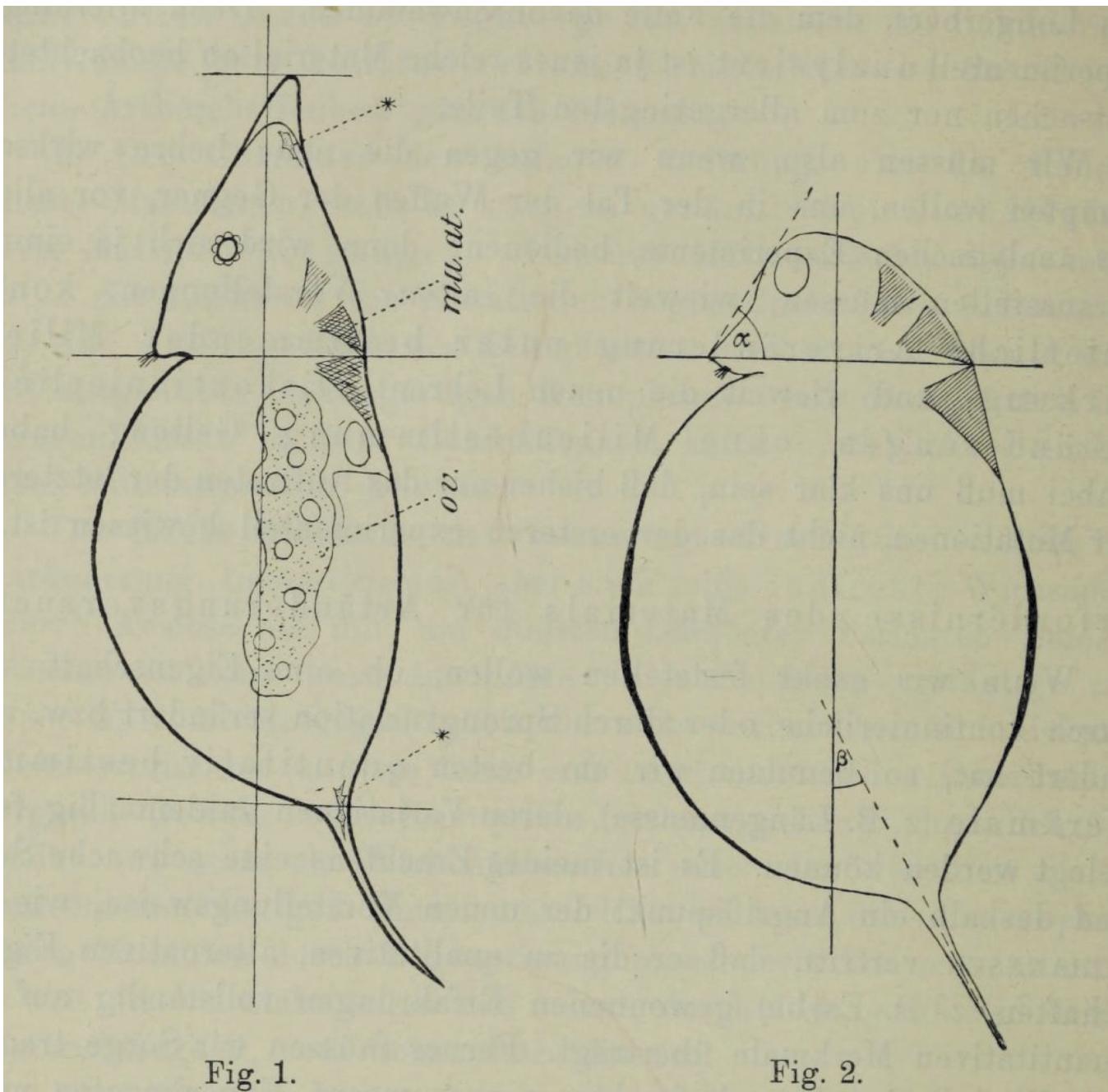


Fig. 1.

Fig. 2.

Fig. 1. Side view of *Hyalodaphnia cucullata*-♀ of intermediate height. The superimposed axes allow the determination of head height. One obtains a single useful "head-base" by drawing a line connecting the point of the rostrum (bottom edge) to the border between the second and third antenna-muscles (indicated *mu. at.*). The midpoint of this line yields a perpendicular line describing the body's long axis, along which we can measure head height and the length of the carapace as shown. Out of these individual measurements one can compute the "relative head height", expressed as a percentage of carapace length. It is necessary each time to draw from a photograph, in order to quickly and accurately capture the outline (including the head-base) on the slide, while at the same time preserving the life out of water of the animal in question. Fig. 2. *Daphnia longispina* from the Lunzer Untersee [trans.: an alpine lake in Austria]. Angles of the axes indicate the facial angle (α) and the spine angle (β).

ation, specifically if the same conditions are used year-to-year. This result shook the foundations of research of statistical variation and heredity, as practiced by the Galton-Pearson school. Specifically, the meaning of the famous regression laws, which to many were the basis of all exact heredity and speciation research, was called into question by Johannsen's findings.)

With Daphnia, obtaining elementary types is easier on one hand, and one the other more complicated. Easier, because most studied natural populations (inhabiting ponds and lakes), are found to be consistent and homogeneous. In the same body of water, in a more than square kilometer pond by Torgau, I could find two Biotypes in an apparently homogeneous population of *Daphnia longispina-galeota*. These types showed not-insignificant differences in their "relative phenotypes" and inheritance. Many bodies of water have yielded similar results, so I assume that it is the same for the Danish populations of Daphnia studied so exhaustively by Wessenburg-Lund.

Thus it is not surprising that in a single lake multiple elementary types of *Daphnia longispina* and *cucullata* coexist, but rather that only one apparent biotype of each elementary type or subtype exists. And therefore we must confront the complicated side of our question (compared with the bean and barley biotypes maintained through selfing), concerning the poorly-illuminated basis of the identity of types.

In considering Daphnia types, in which sexual reproduction and diapause (dauer stage) play a greater or lesser role, we would expect that the mixing of local forms is substantial. In reality, we observe essentially no type mixing. In the case of such mixing, we should always encounter inconstant forms of variable descent, rather than the striking constancy which the forms show from year to year.⁴

The mixing of local types with strange biotypes appears much less important than the mixing maintained within some local types at the end of the breeding season. Through this general admixture within individual germplasms, the differences within the local types will be balanced out as generations proceed.

The transition between obligatory sex and total parthenogenesis takes at least a year. With most Daphnia the lakes show a greater or lesser proportion of the ♀♀ are maintained as parthenogenetic, while the other individuals (majority or minority) go over to the production of dauer or diapause eggs and ♂♂.

It would be worthwhile also to make a comparative study of the means of inheritance in varieties with different degrees of sexuality. In particular, it would be good to bring up those populations which have given up sexuality entirely (for instance, *Bosmina* and *Daphnia longispina-hyalina* of the great alp lakes). In the case of these last forms we must expect the population to be split up into more biotypes.

For the task at hand, these considerations are of theoretical interest (in the selection and orientation with respect to the working material), not necessarily practical meaning. In each of my cultures I deal with single biotypes, because if I have selected a lone ♀, the following generations will

necessarily be parthenogenetic. These generations represent "clean lines", like those that Johannsen studied.

They are thus the first instance of working material for the experimental analysis of the quantitative character, whose change (whether continuous or discontinuous, and whether with or without determination from the milieu) we should study.

Research Plan

The success of such an undertaking requires a systematic approach. It must be carried out in such a way that the local forms compared represent elementary types and not only slight variations of the same type, requiring the following tasks:

1. Analysis of the collected modifications that the various characters of a given biotype can show, which are thus caused by the conditions of the milieu. We will thereby understand the various potential forms (the "Phenotypes" of Johannsen) of each character on the one hand, and on the other define the heritable "genotypic" differences between biotypes, in order that we may compare these biotypes under like milieus. To eliminate the various influences on individual cases of embryonic development, we must not compare individual measurements, but rather the averages of a rather large count of age-matched individuals of the same generation and milieu.

With that said, this first task of analyzing available biotypes depends upon our ability to focus on a single character or trait (for instance, head-height), in three sub-tasks of increasing difficulty:

- (a) Exact determination of the range of variation of the relevant character under all possible conditions of the milieu.
 - (b) Exact determination of those conditions of the milieu, which elicit individual modifications of the character in question.
 - (c) Exact determination of the (heritable) differences, which are shown in the collected modifications of the relevant character between different biotypes under the same conditions.
2. After these initial steps, a second area of work constitutes the testing of thus-determined genotypic differences of two elementary types through crossing. This is to answer the question of whether the ascertained quantitative variations in characters resolve into intermediate forms, or whether they follow (and according to which rules) the rules of dominance and segregation.
 3. Further, we must observe whether biotypes isolated from clean lines can give rise to extreme variants under extended selection.
 4. Finally, we must test whether and how far the continuous action of specific conditions of the milieu can achieve the change of a character within a biotype.

My experiments have been carried out at this point over the course of three years and concern themselves more or less with the set of tasks under the first heading above. I have

⁴see my paper Verhandl. 1908, p. 239.

also made a beginning of my attack on the remaining questions, but unlike the work presented here, they have not yet achieved clear and final results. An extensive description of my attempts will appear as soon as possible in book form, together with the data itself, laying out my underlying interpretations and conclusions.

Observations on crossing and selection in *Daphnia*.

Concerning points 2 and 3 (but little treated in the above enumeration) I will make a few observations here.

Concerning 2, the attempts at crossing have run into obstacles related to methodological difficulties in obtaining perfect data, specifically in generating a sufficiently large number of progeny to obtain average values of the quantitative traits among mongrels. And indeed, whether these progeny are equivalent, e.g. the same culture and generation giving rise to equal males and also perfectly equivalent females. We will therefore see in the following results that the determination of the genotypic values of the quantitative characters as well as the definition of the 'grades' of the milieu, and also the count of the generation cannot be gone without.

The relatively few mongrels which I have obtained from numerous experimental crosses between *Daphnia longispina*- and *cucullata* local types show intermediate characters, but an unambiguous evaluation is at this point, as written before, impossible. I address these points here only to alert other researchers that the inviting mongrelization-experiments (at least for quantitative characters) will only yield value from an extensive phenotypic and genotypic analysis, and then only if the obtained material is adequate to the questions under study (average values, the equivalence of the parent stocks).

Concerning 3, the large majority of my experiments attempting to call forth extreme variants under selection upon clean lines have yielded negative results. This is very similar to the results of Johannsen. On the exceptions from this rule, and on the various experimental series where changes have occurred, I cannot at this point provide much clarity. Therefore, in the following pages I will only touch upon these points so far as I believe will serve future researchers.

I will briefly consider the observed mutations, through whose isolation (selection) one can obtain what are actually strongly changed heritable lines, in another context (Seite 137). They are certainly of immaterial meaningfulness in terms of the generation of types in *Daphnia*.

Now, the important question concerns whether a continuous character can be changed only through continuous selection on variants (those increasing or decreasing it, for instance). I wish to emphasize the necessity of clearly separating the influence of the selection from that of the milieu in changing this character. This proved quite difficult in *Daphnia*; it seems doubtful whether factors of the milieu (including during embryonic development) can be entirely eliminated in the study of selection. Instead, the promising studies are those in which selection factors can be determined through, for example, comparing the effects of different mi-

lieus on unselected populations to age-matched individuals who have been selected for the strongest deviation in the character. The influence of selection then appears out of the difference between the simple and the combined methods. [translator's note: ?] However, we have not yet come so far. We will see that one type-changing milieu (of four) at this point has only the first beginnings of something to show, considering so far as the artificial evocation of new genotypes. On the selection of new variants, consult Seite 158.

However, as we will see next, from the analysis of the existing *Daphnia* genotypes we may already have arrived at compelling conclusions about the nature of the variation of types.

I. Exact determination and analysis of the collected phenotypes of the quantitative character "head-height".

We understand "phenotype", like Johannsen, to signify the average value of a character under some circumstance, for instance the middling length of a stalk in an evenly watered and manured field of rye. This phenotype can be singular, if all the plants belong to a single elementary type, or only apparently singular, when more heritable ("genotypic") different elements are planted, when we deal with a mixed population. However, in the following work we concern ourselves solely with phenotypes whose singularity can be guaranteed through previous hereditary experiments. To simplify the picture further, we prefer to focus on some particular character, namely the head-height of *Daphnia* land-races (which we treat as elementary types). Further, we obtain the 'relative' head-height by also considering the length of the carapace (compare Fig. 1 and relevant explanation).

Our first task is now to compute the phenotype of head-height by measuring age-matched samples of 100 examples (♀♀ or ♂♂) from natural populations, from which we obtain a frequency curve and can then compute the desired average (phenotype).

Such a calculation would only have worth if we are able to obtain age-matched animals (that have, for example, gone through a like number of molts), as they might also change their relative head-height over their lifetime. We must confine ourselves in this analysis to roughly similarly "grown-up" animals, preferably considering only the ♀♀ , for the purposes of constructing our curve. We may nonetheless in many cases obtain multimodal curves, if for example adult ♀♀ from different generations are available, that can vary in their head-height in the early year particularly.

It is therefore not permissible to compare the "natural" frequency curves with curves generated from cultivated materials, so much so that we will never make comparisons involving individuals that are not from the same generation and age class.

If we perform a similar experiment in wild animals at different times of year, across many *Daphnia*, we reliably find quite different head-heights in spring, in summer, and in autumn/winter. Thereby, the frequency-curve and its mean continually move; we obtain a spring curve, a summer curve, *et cetera*. Or more precisely stated: the curve (and with it,

the phenotype) of head-height shifts with time of year from Spring until September to the right, only to return once more to the left (Fig. 3).

Wesenberg-Lund has described the change of head-types across many Daphnia of the Danish lakes, giving us a characteristic example of cyclomorphosis. These samples of animals are of course taken directly from the lakes in question, not from cultures. These observations are thus very informative, collected as they are across many years, in descriptively characterizing the forms of local races. However, in seeing beneath the description to the particular heritable nature (the genotype) of an elementary type, we cannot do without cultures.

In our cultures, we can artificially regenerate all of the different head-heights that we observe in lakes over the course of a year. We must, of course, take care that our culture conditions⁵ be correct and appropriate. I have accomplished this in various attempts to obtain pure pelagic *Hyalodaphnia cucullata*.

a. The causes of different head-heights.

There are three different principal factors from which the different head-heights in the space of a Biotype are derived (similar to other characteristics of form, such as spine length, spine angle, brow angle, and overall size): feeding, temperature,, and generation number.

External factors Until this point the role of temperature as a biological factor, at least since Wo. Oswald's⁶ treatment of the subject, was thought to be related to its inverse relationship with water friction (at higher temperatures friction is lower). Consequently, planktonic organisms require a higher buoyancy at higher temperatures to remain in suspension than they require at lower temperatures.

The buoyancy of Daphnia bodies is apparently increased by, among other things, the body processes (on one side the head-height dependent "helmet", and on the other side the mantle-spike); or also by reducing overall size, which increases relative surface area. The hypothesis that relevant body alterations may be triggered or caused by temperature changes is therefore obvious.

However, my cultures show to the contrary that temperature plays only a secondary role in these alterations. Further, head-height is not altered through changes in internal friction. In coming to this last conclusion, I rely primarily on results from the addition of quince mucilage to the medium, which is better borne by the animals than supplementation with glycerin or salt. Considering that the result was always negative (apart from a definite degeneration of the older cultures in higher concentrations), it would not be profitable to further explore the details of these experiments.

Increases and decreases of the temperature itself are in contrast to these results, as Wo. Ostwald very rightly observes (though he does not interpret them properly), show-

⁵Consult my note on the rearing of Daphnia in the Intern. Rev. d. ges. Hydrobiol. 1908, Volume 61, 871-874.

⁶From his numerous writings on this subject, one is especially relevant: "Exper. Unters. ueber den Saisonpolymorphismus bei Daphniden" (Arch. f. Entwicklungsmech. 1904).

ing a noticeable influence on the head-height, in addition to an effect on overall body size.

We shall consider the latter, simpler alteration first. It is known that body size can in fact be reduced by heat, but only when holding constant a poor to middling diet. With more food, in contrast body size is absolutely not reduced by heat, but rather considerably increased.

The connection here is obvious: under higher temperatures bodies require a larger amount of food for their higher vital activity (movement, heartbeat, and fertility are very obviously increased). Therefore, they must devote less material to their growth, with only a meager quantity of food at their disposal. If in contrast food is available in surplus (which is unlikely to be the case for the pelagic types, which sustain themselves upon all available detritus, Bacteria, *et cetera*), then warm conditions induce an increased capacity for assimilation and with it an increase in body size.

The same principle applies also for head-height or the "Helm" of the animal, as shown in numerous important experiments upon this important point using *Daphnia longispina*, *galeata*, and *cucullata*. I will not go into these experiments in detail, and only share that I have achieved greater head-heights in my cultures with low temperatures and rich diets of *Chlorella* than I have with high temperature and poor diets. Specifically, after a week-long incubation in a thermostat at 30°, the heads stay small if there is not a rich algae diet (consider that the "natural" temperature ranges from 18-25°). Even at 6-10°, I can achieve higher heads with rich food than I can with lesser nourishment at 30°. At temperatures under 5 or above about 32°(the bounds differ between biotypes) metabolism is quite poor, such that past these points animals 'stunt' or die.

The smaller heads that overwintering *Daphnia longispina* or *cucullata* show in the colder seasons (Wesenberg-Lund was the first to show that they are all alike) are attributable on the one hand to lesser food, and on the other to a degradation of the assimilation capacity by cold. The high helmets of the summer, however, are achieved through increased food; or more exactly, through the consequently increased assimilation. (Concerning the other innate factors effecting helmet size, compare page 143 below).

The subject presented here is too complicated to be immediately handled in detail. What we need immediately, however, we can abstract: the most essential factor of the 'milieu', the living situation of our Daphnia. It is particularly important that the quantitative differences of the assimilation be directly proportional to the quantitative differences of tested characters, while a second trait (sex, page 144) is held proportionally constant.

It is methodologically important that the influence of temperature, the second factor affecting assimilation intensity, be easily disabled in analytical experiments. For this, one may simply take care for the warming of the cultures. The same may be said of the total state of the 'milieu' of the cultures: salt and gas content, volume, and light in adequate and equal amounts, such that the single factor of food supply is left, and this last is well-measured (see appendix 1, page 166).

We can thus establish the supplied quantity of food as the

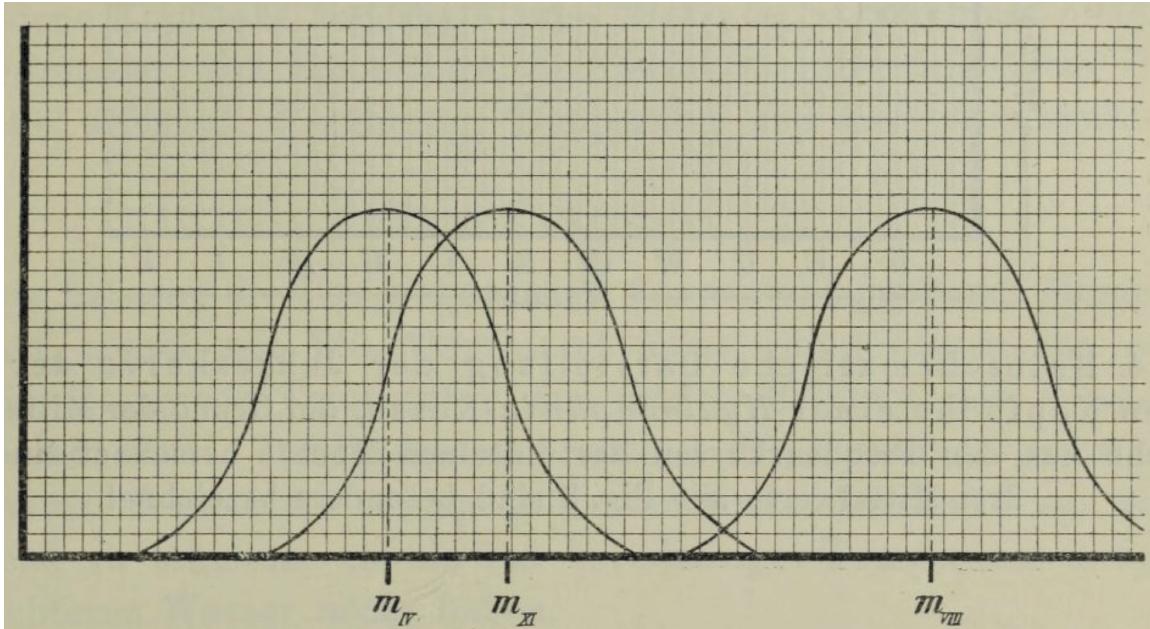


Fig. 3. Semi-schematic representation of three frequency curves of relative head-height of certain *Hyalodaphnia* races collected from the wild. The horizontal axis represents a measure of relative head-height, and the vertical axis represents the percentage of individuals with a given head-height. The left curve represents grown April-caught ♀♀, the right August-caught, and the middle November-caught. For each of the three curves, the mean is indicated with m_{IV} , m_{VIII} , and m_{XI} , in turn indicating the number of the month.

measurable cause of head-height particularly. Similarly, we can ignore the quality thereof if we feed comparable cultures with the same pure cultures of *Chlorella* (or some other algae) in measured amounts.⁷

Innate factors We must now put aside nutrition (and the secondary factors of temperature and so on, that we have eliminated through experimental treatments) to take another variable factor into consideration, namely the influence of the generation number. This factor is a part of the residual constantly-working heritable helmet-potential, whose existence we will shortly observe in total. We saw above that assimilation intensity (as a product of nutrition and temperature) must be particularly crucial in reshaping both the body plan and the head height. From this we may clearly understand this special assimilation process, and we might say that this operation of nutrition on the helm-substance is directed by inner forces, which are in truth the decisive factor. This is, namely, the inherited potential for helm-growth. We can understand this potential as acting on available materials in

⁷Unfortunately it is often not possible to perform immediately consecutive experiments. This makes it rather uncomfortably noticeable that nutritive value of these algae fluctuates in ways that are difficult to understand (perhaps together with their variable tendency to stay suspended in water or sink to the floor). Especially, care must be taken that food algae must not be grown next to Bacteria or other algae, which often (for instance with the spiked Raphidia) render the algae unpalatable to Daphnia. In such cases one may obtain totally untrustworthy results.

the hypodermal cells of the head; or better, as an arrangement of these materials, which reacts to the certain qualities and quantities of nutrition (blood) through the construction of a ridge-like anterior process of a certain height.

We now approach what seems to me an especially important point, so it will be beneficial to briefly consider the morphological and physiological components of this helm-growth. The critical region of the Daphnia head is normally found by comparison to the chitin-covered hypodermis, which is thickened on the rest of the body. When no helm is present, this has something of the form of a roof. Invariably (except in the case of very low heads) this roof shows a sharp ridge; the systematists say that the head is 'keeled'.

The thickened hypodermis underlying this ridge or keel is the seat of the 'helm-potential', whose nature we would like to investigate. The hypodermis can remain low and compact throughout life (as shown by Fig. 6a), it can however also grow out to a high and narrow protrusion (Fig. 6b), which then is covered with a thin layer of chitin and takes on the appearance of a helmet when viewed from the side.

In reality the 'helm' is a narrow blade; the young animals in the mother's brood compartment maintain it, so long as their chitin is not yet hardened; in such close spaces it resembles a sleeping cap turned down to the left or the right.

But how does the stimulus direct development at this point of helm-forming potential? This may occur by two quite different ways, depending whether it acts upon the helm-

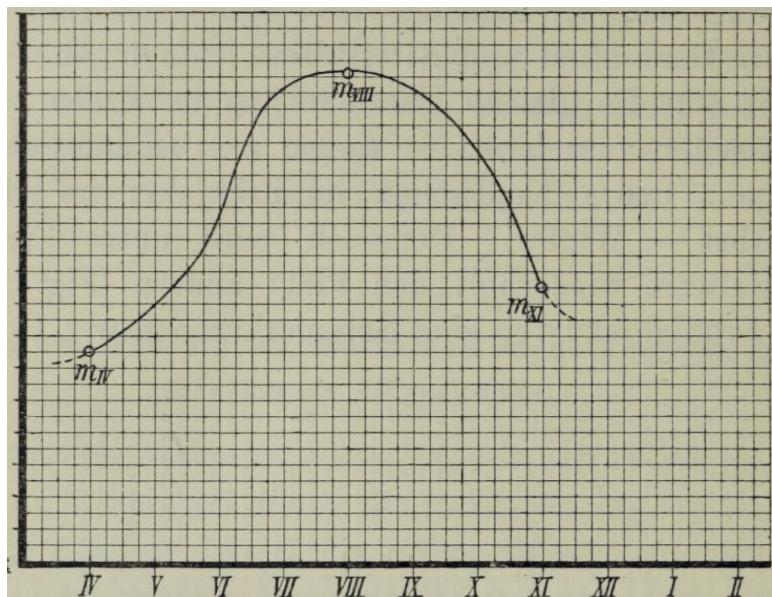


Fig. 4. Semi-schematic representation of the change in relative head-height of a *Hyalodaphnia* race, based on means calculated from the frequency curve of each month. The vertical axis represents a relative head-height measure, the horizontal access indicates month of the year (as numbered in Roman numerals). Three means from Fig. 3 are indicated.

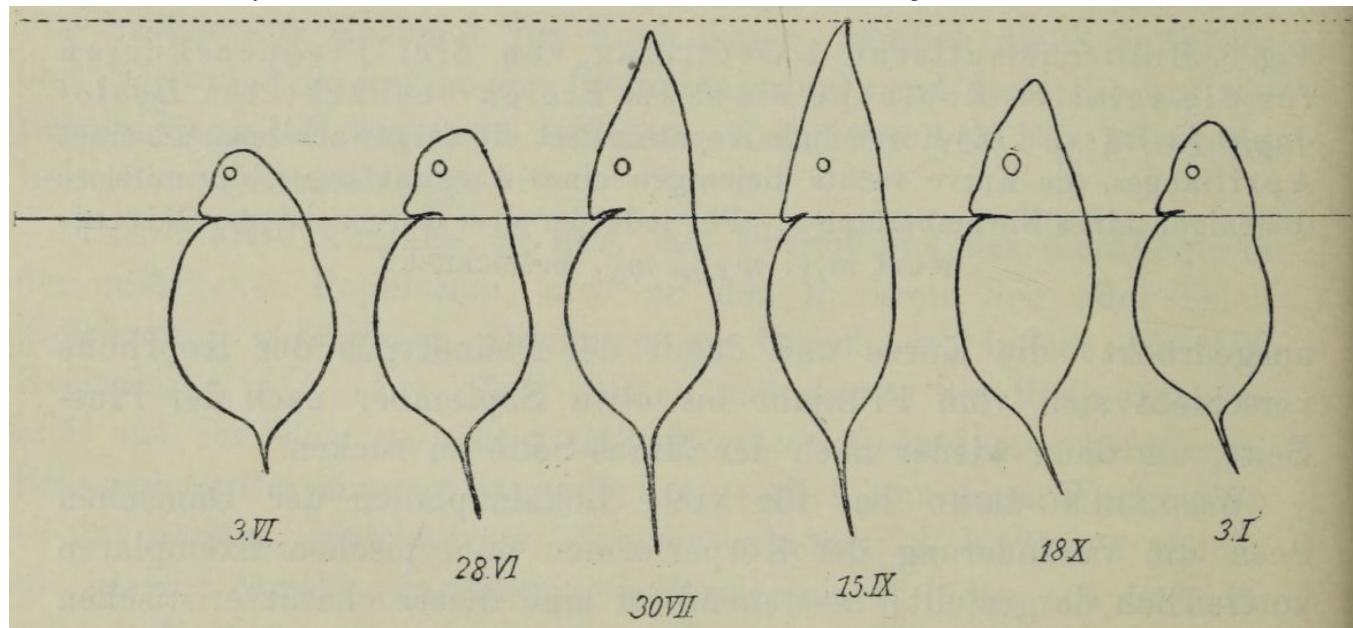


Fig. 5. Representation of the cyclomorphosis of a *Hyalodaphnia*, after Wesenberg-Lund. These are likely single typical examples of large similar populations, drawn next to each other. (By joining the vertex points one may obtain a year-curve of absolute head-heights, which however is not so useful as the means of relative head-height used in Fig. 4. The examples used by W.-L. for illustration (Plankton-investig. of the Danish Lakes II. 1908) from Skanderborg Lake were chosen because they showed a definite periodicity in both spine-length and spine-angle across months.)

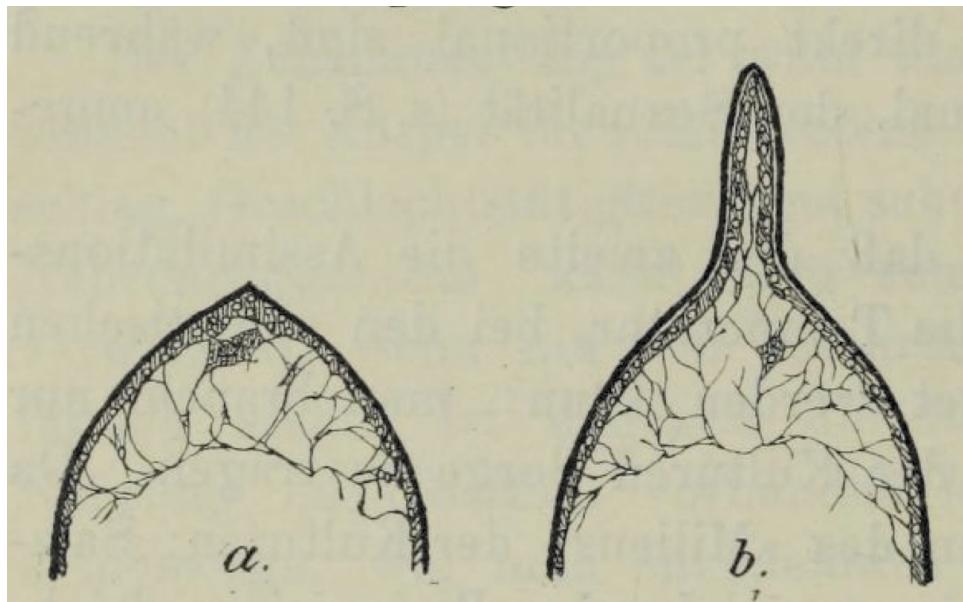


Fig. 6. Two frontal sections through the crown of a *Hyalodaphnia*, conducted approximately in the plane of the 'long axis'. Chitin in black, hypodermis cross-hatched with white inset kernels. (Underneath branched connective tissue single large amoeboid cells is shown.) *a*, Helmless animal (as Fig. 5 at left); *b*, Beginning helm growth.

building cells of an embryo or of an adult animal. Namely, in the latter case, this cell mass retains the potential for development throughout life (or at least until the 'advanced age' of 10-12 weeks). In younger animals the reaction of course proceeds much faster and more completely, than in the case of older examples.

In the embryos the sustenance that mediates the stimulus approaches the growing cells by means of an external source; this is the nutritive juice in the brood chamber of the mother. Higher or lower concentrations of this stuff is that which chiefly determines whether the young animal receives a high or low head. This juice is familiarly thought to exude from a cell layer on the floor of the brood chamber, where it itself is obtained from the relevant nutritive pool of the mother's blood. Additionally, the yolk content of the embryo appears to contribute to embryonic or youthful helm formation.

In the second case, of the formation of the helm before the molt of the grown animal, it is the greater or less nutritive content of the blood plasma that acts as a regulating stimulus. Furthermore, the activity of amoeboid cells comes into play, which are found at the locus of helm development (and also at the base of the mantle spike) in the form of a small plaque. This is likely composed of 'blood cells'; their activity can tentatively be identified with the influence of the blood upon the helm or spike development. See Fig. 7.

Unfortunately the processes of assimilation are researched little or not at all in these Entomostraca [trans.: an outdated taxonomic category of crustaceans.] (see part 2, S. 168). One may however learn something of this process by means of the complete transparency of these animals. If given rich food, a heretofore malnourished Daphnia will

very quickly begin to show characteristic alterations. The gut will immediately fill with algae, and one can then measure the time until the appearance of the resorption droplets appearing in all of the gut cells. Then follows a cumulative alteration of the heretofore unseen (because totally water-clear) plasma into a visible juice, at first yellow and then gradually rather opaque. From this blood juice the whole body is provided with a rich stream of nutrition. In a surprisingly short time (at permissive temperatures), one now observes the previously invisible gonad swell, and the previously nearly fatless body becomes fat, appearing with its characteristic red or violet color. The action of the enriched bloodstream on the helm-developing cells is similar: the cell layer increases through imbibition and perhaps also increased number of cells, and by the next molt the head height shows a substantial increase. Conversely, one can decrease the head-height through a molt by starvation; this has been described by Wesenberg and others. Even for some time before the molting, the new smaller helmet Anlage is embedded in the earlier one, such that the two contours are observably separated (Fig. 7).

We can finally state that, at a given temperature, each quantity of food corresponds to a specific increase of the head height, even as it corresponds to the building-up of fat bodies and ovaries. For each biotype, there are set rules for the allocation of available surplus (as absorbed and dissolved in the blood) to each different region or organ of the body, and to the increase of energy production (heart rate, swimming motion). For example, there is pronounced competition between the ovaries and the helm-building cells. In each case, the severity of the 'war' between ovary and helm depend upon many aspects of the assimilation process (qual-

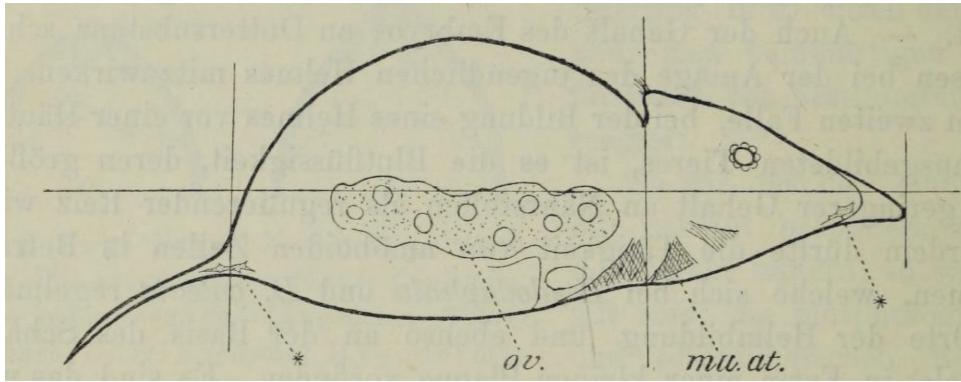


Fig. 7. *Hyalodaphnia*-♀ shortly before molting, with diminished nutrition (or assimilation). The contour of the new helm is already visible, it is already become substantially diminished. * indicates plaques of amoeboid cells (blood cells?), which play a role in helm and spike development. *ov* ovary. (Compare also the explanation of Fig. 1.)

ity of food, temperature, etc.), on the special character of the biotype in question, and most importantly on the generation number.

In the ‘generation number’ we have a ‘cyclical variable’, which is to say specifically, a successively diminishing role of an innate factor in helm development. This inner factor, the ‘helm-potential’, reacts in different generations with different intensities to the same fixed milieu. By this I mean nourishment and temperature and therefore assimilation, which we previously learned to be the regulating causes or regulating stimulus of helm development. I noticed that the first generation reached a markedly smaller average head-height compared to later generations, across many forms of *Hyalodaphnia cucullata* with similar nourishment etc. Further, for two local biotypes of the same species I can state that the middle generations reach a higher head-height than the later, in a similar milieu.

Here it is worth noting that the early and late generations of these elementary types normally occur in the spring and in autumn, when in the wild they can develop only an inferior assimilation intensity relative to those in the summer. Thus other factors of assimilation are noticeable, in addition to the regulation of nourishment quantity by temperature.

With these, we have learned the innate factors of helm development: the innate cyclically diminishing helm-potential over generations; and on the other side the external factors: first nourishment, second temperature, and further the gas and salt content and other qualities of the water. From all these various external factors results the dramatic variability of assimilation intensity, to which we have learned to attribute the similarly variable head-height. Otherwise expressed: the assimilation intensity acts as a graded stimulus, that releases a corresponding intensity of reaction in the substrate of the helm-building cells. This reaction leads to specific heritable helm-potential in this same substrate.

b. How do we determine the collected phenotypes of head-height for an elementary type, and how do we represent them?

We desire to study the development, inheritance, and changing of these specific helm-potentials. From these studies, we desire to see whether the position occupied by De Vries and Johannsen — change of the quantitative trait only through mutation and determination by the milieu — is supported or not. Through our knowledge of the helm-building factors, we are now in a position to make determinations regarding the causes (via induction or regulation) of all possible head-heights independent of each phenotype that this character depends on, for each elementary type that we investigate.

Without giving all the figures independently that were found for different biotypes, we wish to briefly consider how one may conduct an analysis of the phenotypic entirety⁸ of a character. Further, how may one best represent this analysis to lead to a solution of the question we have posed?

To simplify this general representation, we confine ourselves to the triggering cause (assimilation intensity) related to the factor of nutrition, and attempt to isolate the graded effects of this factor for study. We will leave aside the lesser causes of temperature, light, and water quality, by considering only cultures in which these factors are held constant and only the quantity of food is varied. (A study of such cultures in which nutrition is constant but other factors, such as temperature, are held constant, would not be novel. Rather, it would arrive at the same goal by an alternate path.)

Moreover, we observe only the effects on ♀♀ and leave ♂♂ alone. Finally, we restrict ourselves to only three levels of nutrition: poor, middling, and rich. Our task is to determine the phenotype of head-height for each such level.

To achieve precise nutritional quantities, we use carefully filtered water with certain quantities of *Chlorella vulgaris* (or similar algae: Palmellaceae *Kirchneriella lunaris* or oth-

⁸The ‘collected phenotype’ (*Gesamtphenotypus*) of a character we may unfortunately not say, because Johannsen has defined this term as the sum across characters of an organism, for instance their average in some particular condition.

ers) added back from pure culture. We must replenish their quantities daily to avoid depletion by transferring the animals, because otherwise *Daphnia* will reproduce due to favorable conditions, and likewise, algae will fail to reproduce under unfavorable conditions. This redeployment of animals can be effected with a pipette, or for large cultures it may easily (and with practice speedily) be carried out with silk gauze. For "poor nutrition" conditions, one provides enough algae to sustain life, and also to allow reproduction; though only one or two eggs are desirable. The "rich nutrition" is attained by supplying algae in excess, and is immediately recognizable by the green color of the cultures. In this greening and the overabundance it signifies, one still finds impairment of some *Daphnia* (decrease of head-height and general vitality). Otherwise, in this thick, green-yellow algae water do well, and it is only here that the maximum head-height, body size, and speed of movement are found. (More curiously one sees such natural forms in the animals that live in pelagic zones of clear lakes, for instance *D. longispina* of the Lunzer Untersee). "Middle nourishment" lies in between the empirically determined minimal and optimal quantities ("poor" and "rich"). The correct input of algae is somewhat subjective, and is assisted by the quantity of *Daphnia* in culture, and is therefore a retrospective designation following experiments considering these factors. The method by which one may know the phenotype or mean value of a character for a certain life stage is well-known enough. One uses a large number of measured individuals to construct a frequency curve ("Galton curve") or, as is simpler in some cases, a distribution schema. From one or the other of these one reckons the mean value of the relevant character.

This mean value expresses the following: for the collected individuals of the relevant li in these cultures, which are essentially identical (see the first appendix), the "normal nourishment of the eggs and embryos in the mother animal [trans. note: note that in this case "normal is not a synonym for "Gaussian"]". The higher variations owe their higher head above all to accidental chance, that an abnormally small number of eggs fully matured in the given milieu. The lower variations owe their deviations similarly to too great egg production all at once. However, generally the egg production of age-matched ♀♀ raised on similar milieus are rather constant. The middling deviation (variability index) is here in most cases not meaningful, because in this case it gives large differences and complications even within the same Biotype, which we must prefer to neglect. Figure 8 shown here represents no specific instance, but rather a schema for the head-height frequency curves that we obtain for *Hyalodaphnia*.

We can draw the three helm-height frequency curves for poor, middling, and rich nourishment in the same coordinate system (Fig. 8), and with them the three corresponding mean values m_1 , m_2 , m_3 , the three phenotypes.

In overview, let us once more represent the dependency of the same phenotypes on the conditions of life (nourishment) through the following graphical representation. I hold on the abscissa axes the nourishment quantity, from zero on the left to overabundance on the right. As the assessed value, one may use either 1 cbmm [Trans. note: probably cubic millimeter] of an intensely green Chlorella [naehrloesung] or 1

small platinum loop full of Chlorella from an agar culture. Both quantities are agitated in a certain quantity of water (that differs between species). A mathematical exactitude of measuring this dose is obviously difficult to obtain, and unnecessary.

From this abscissa axis I have raised to each single point an ordinate, which represents the mean value of head-height derived from the frequency curve, to which the nourishment quantities correspond. [Trans. note: This is confusingly phrased. In modern parlance, Woltereck has created a density plot. In his time, this was not so common a procedure, perhaps leading to confusion. Alternately, I may have missed his meaning.] When I connect the end-points of the ordinate, I obtain a curve (Fig. 9, 12) which I will describe as the "Phenotype curve". The curve constructed thus can be applied to some quality of the water (temperature etc.) that is shared between all cultures.

If I want to represent all the phenotypes of the analyzed character, I must next fill in all the relevant temperatures in my coordinate system of the phenotype curve, or at the for low, middle, and high temperatures. Through this I would obtain three phenotype curves of the same character for each elementary type, the ratio of whose positions against one another will be somewhat different, as determined by heritably fixed differences between Biotypes.

Further, I must distinguish between the first, middle, and late generations (computed from dormancy onwards) for some elementary types. The form and the relative positional relationships of these curves are also heritably fixed for each Biotype.

Upon such analysis, the different generations of some Biotypes not only show different reactions to the provided food quantities, but also to temperature differences while holding food constant. It is therefore necessary to consider three temperature curves for each generation.

Finally, the curves for the helm-height of the ♀♀ and the often quite different ♂♂ animals are actually separated from each other. The ♂♂ animals of two elementary types can hold themselves totally apart in these points, whereas the ♀♀ animals show great similarity (for example the colonies from Frederiksborg and Moritzburg).

However, to attain a tolerably complete representation of the phenotype regularities, it would "suffice" for such a quantitative character as head-height to fill in the already not-insubstantial count of nine phenotype curves, namely for the control of the ♀♀ in the following combinations:⁹
 Beginning generations for low, middle, and high temperature,
 Middle generations " " " " " "
 Late " " " " " ".

Now, each phenotype curve of the analyzed characters is based upon the mean values of at least three frequency curves, for poor, middle, and rich nourishment, so for approximate knowledge of the specific reactions of this single character we must use no fewer than $3 \times 9 = 27$ cultures and from them obtain frequency curves and their related middle values.

⁹The following orthography follows Woltereck in some detail.

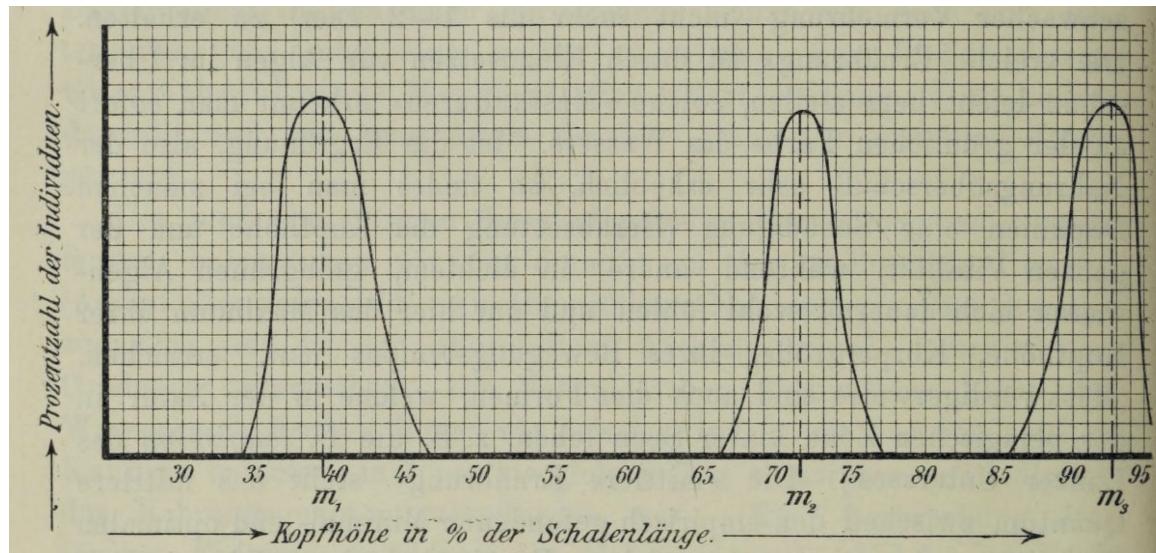


Fig. 8. Semi-schematic representation of the frequency curves for the relative head-height of a *Hyalodaphnia*-race, under minimal, middling, and optimal nourishment conditions, with the corresponding mean values: m_1 , m_2 , m_3 . Abscissa label: Head-height as a percentage of the mantle length. Ordinate label: Percentage count of individuals.

We must here neglect to go through the particularities of the number relations. In this instance, it comes down to clarifying the coming into being of such quantitative characters as follows: the specific head-height of a given elementary type is heritably controlled through a large number of relations.

Even with these 27 related values, we remain quite far from an exhaustive definition of the specific helm-height and helm-potential of a tested elementary type. On the one hand we have left out all the intermediate values between the three chosen values for each of the helm-building factors. On the other hand, we have left out specific differences due to external factors, gas and salt content of the water etc., and also the effect of intrinsic regularities. Examples of such intrinsic regularities include the correlation between helm-height and germ cell proliferation and the development of head-height from molt to molt. We may file also with these factors other particularities of the apparently simple head-height and its heritable basis.

Now, overall we have very little reason to see head-height as especially complicated in the regularities of its relationship to environment relative to other bodily organs. A more exact analysis of each quantitative character may yield a similar richness. The analysis of "helm-potential" is merely more readily measurable to prosecute than the peculiar reaction intensities of the burgeoning of the ovaries or the fat bodies, the eyes, and so on. (At the least, we will later (s. 144) approach sexuality more closely, as an entirely different sort of character in its corresponding relations.)

We have hardly any reason to expect a principally different nature of quantitative character potentials in other organisms. For example the length of beans in Johannsen's biotypes would be such a character, whose potential (gene) would be found to be related to specific relations of the

milieu, even if we manage to test the single influences of its circumstance (as in *Daphnia*). Regarding the quantitative traits (coloration etc.) whose specific differences appear much simpler to the casual eye than those described here, I will not venture anything. Different sides of the argument have already confirmed that they cannot be principally different from the quantitative traits and potentials.

And so, what have we achieved so far?

The understanding that any such quantitative character in its wholeness can only be fully understood and characterized by a great horde of relations (i.e. the relationships of reaction intensities to trait variations from stimulus intensity to trait variations from nutrient assimilation). The whole of these relations may be represented only through a number of data tables, or more comprehensively through the 'phenotype curves' that I have put forward for this purpose. If we want to realistically analyze the importance of these various specific relations, the number of these curves is nine for our cursory evaluation here, and will grow to an endless quantity from there. The totality of these relations, described in these curves, we can call the **reaction norm** of the quantitative character in question. We can quiet this cacophony somewhat to the slighter number of phenotype curves we actually have, which in praxi we must do. Many times over we will have to decide to construct a phenotype curve only for the "middle generation and a "middle temperature. We can then also from these curves speak of the "partial reaction norm" (Fig. 9) of the corresponding character. We may however go no further in our claims in these cases: we cannot take as given any measure of a quantitative character for which for which we have not at the least measured the same (in this case, middle) grade of nourishment (or assimilation).

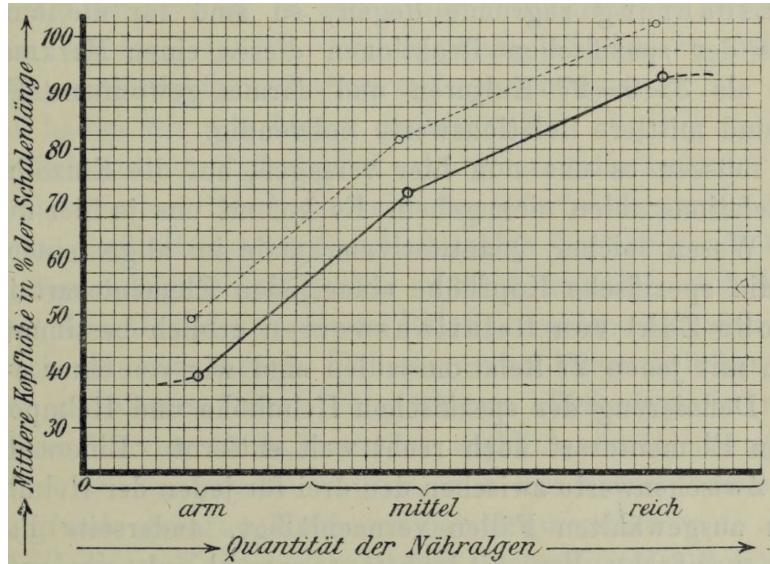


Fig. 9. The three mean values of Fig. 8 represented as the simplest “phenotype curve” (black lines). The animals represented belong to a middle generation, cultured in middle temperature.

c. What can we infer from the genotypical basis of a specific head-height and the genotypical difference of this character for two elementary characters?

The foregoing illuminates that we can define or show support for an elementary type only through the specific and relative reaction norms of its different characters.

We interest ourselves in these facts not only because of their consequences for the systematics of plants and animals, but rather principally for their import for the sciences of heredity and speciation. To familiarize ourselves with this importance, we must recall two indubitable and eternal truths. First is the proposition that everything that is specific to an elementary type or a variety, by which I mean that which distinguishes it from other biotypes, must be bequeathed to the next generation in the germ cells. Second: a new type or elementary type arises through the heritable alteration of any specific character.

The “genotype” (the genotypical foundation) of a quantitative trait is its heritable reaction norm. From both of these propositions the following is very clear:

1. The total reaction norm with all its countless specific relations is inherited as the principle for helm development (helm-Anlage). Therefore, the “genotypical basis” or “genotype” or also the “gene” (all terms from Johannsen, the appropriateness of which we will not speak of here) for helm development are all equivalent to the reaction norm, as we have defined it.
2. The biotypes of *Daphnia* arise and have arisen through heritable changes in the reaction norms of their head-height and their other characters. From these reaction norms the natural question arises concerning our assumptions: are these reaction norms changed only through mutation and without determining influence of the milieu? Or are they continuously modified through the action of changing environments?

We can posit a discrete modification of helm development (if such actually exists distinct from the more familiar continuous variation) only under like conditions of the milieu, and specifically only if in similar conditions of nourishment the head-height appears by some quantity greater or lesser in one type. Such a difference transgresses the boundaries originating in embryonic nutrition and resulting in the continuous frequency curve of the phenotype. Such modifications can in fact occur; I have observed them myself for the rostrum length of *Daphnia longispina* (Fig. 10).

TRANSLATION IN PROGRESS

Translator's Note

It is the unfortunate case that the recent dominance of English in academic (or at least scientific) discourse has consigned many classic papers in other languages to, at best, obscurity. This was brought home to me when I attempted to locate that which you see here: an English translation of Woltereck's famous paper. I was at the time trying to refresh my hazy memory of the idea of the reaction norm, in connection to a project investigating temperature sensing in *Arabidopsis*. I was shocked to learn that not only had no one translated this paper, no one had even digitized it or its journal in a searchable fashion. I was ultimately able to locate the correct volume of the *Verhandlungen* as scanned by the good people at the Biodiversity Heritage Library. As a beneficiary of both the dominance of the Anglosphere and a rudimentary education in German, I felt that I was under some obligation to address this historical accident. I hope that I honor Woltereck's memory with a translation that is neither unnecessary nor poorly executed.

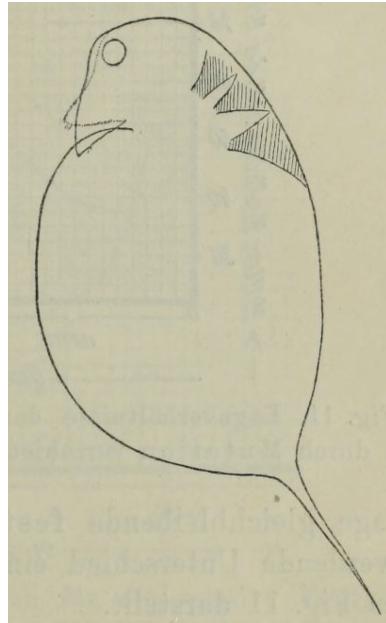


Fig. 10. A heritable, discrete recurrent variation of the rostrum length of *Daphnia longispina* (Lunz Untersee). The original contour of the rostrum is drawn in dotted lines.

This translation is also an attempt at self-improvement in German. Consequently, there are no doubt an abundance of errors scattered throughout the text. These should be attributed to my incompetence and not to Woltereck's inarticulacy. I would gladly receive news of possible improvements that could be made to this text.

I attempt throughout to preserve some of the character of Woltereck's writing. For instance, I have attempted to preserve his repeated descriptions of Johannsen's work as '*bekanntlich*', familiar or well-known to the reader, which is probably no longer true, but which properly captures his veneration. In other places I have used words I judged to reflect Woltereck's usage rather than more familiar contemporary concepts of biology, for instance replicating his usage of 'milieu' when today we might speak generically of 'environment'. However, overall I have tried to emphasize legibility over literality. This being 'science', the medium is less important than the message.

I think that this means of translation also preserves something of the place of Woltereck's work in his time. In conclusion, I hope that my translation helps posterity to remember Woltereck and his contemporaries a bit. The idea of the reaction norm has not yet faded from memory, and indeed its usage has expanded in recent years with the rest of the scholarly literature (Fig. A); all the same, I believe that the genomic era needs constant reminding of this central biological idea, to keep its focus rooted in the organism, and to combat the ever-present ideology of determinism.

Acknowledgments

The digital version of the original article was digitized and hosted by the Harvard Biodiversity Heritage Library. I am grateful to AAAI and various people for implementing the

AAAI style available on Overleaf used for this translation, and the www.dict.cc online dictionary as a general translation resource.

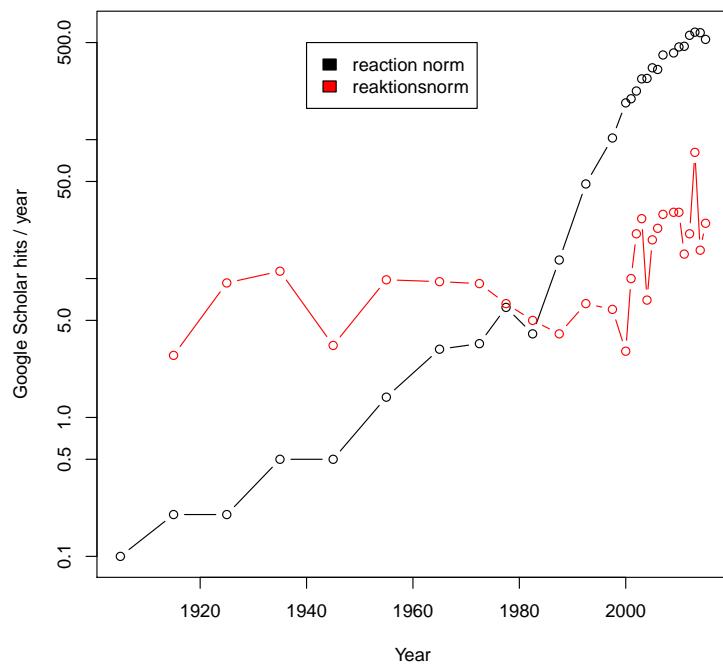


Fig. A. Usage of the terms "reaction norm" and "reaktionsnorm" in the Google Scholar database since introduction of the idea. Due to sparsity, queries for earlier periods are performed across intervals of multiple years to smooth data. Searches performed late 2015, note that numbers for later years may be revised upwards as new documents are added to the database.