Table 1. Cofactor requirements for the biosynthesis of vitamin A. The complete system contained 11 mg of soluble protein (104,000g supernatant), and an equivalent amount of washed cell particles (sedimented between 2000g and 104,000g); 200 μmole of potassium phosphate buffer, pH 7.7; 36 μmole of nicotinamide; 4.8 μmole of $MgCl_2$; 12 μmole of sodium taurocholate; and 10 μmole of glutathione. α-Tocopherol (1 mg) was added in solution in 25 μl of acetone, and then substrate C^{14}-β-carotene (0.7 μg, about 1100 count/min) in 50 μl of acetone was added. Final volume, 2 ml. Fx, fraction (after column chromatography); EDTA, ethylenediaminetetraacetate.

Omission from or addition to complete system	Distribution of C^{14} after 75 min incubation (percent)				
	Fx 1 (β-carotene)	Fx 2 (retinyl ester)	Fx 3 (retinal)	Fx 4 (retinol)	Fx 5+6 (polar; acids)
None	47	1	45	4	4
Minus enzyme*	87	2	5	3	2
Minus O_2 (argon as gas phase)	89	2	3	3	4
Minus bile salt	83	2	9	3	3
Minus glutathione	64	2	25	4	5
Plus EDTA (20 μmole)	51	2	38	4	5
Plus ascorbic acid (20 μmole)	44	2	43	5	8

* Minus both the soluble protein and the cell particles.

ment for bile salt for the conversion of β-carotene to retinal. The requirement for bile salt was relatively nonspecific since comparable yields of retinal have been obtained with sodium glycocholate (6 mM), cholate (6 mM), and deoxycholate (3 mM). Sodium dehydrocholate or lithocholate were ineffective in this system. The system was stimulated substantially by the addition of glutathione and was not affected by the addition of ethylenediaminetetraacetate (10 mM) or ascorbic acid (10 mM). There was no requirement for the addition of reduced or oxidized pyridine nucleotides.

The product of the reaction has been identified as retinal by addition of pure unlabeled retinal, followed by formation of the semicarbazone derivative (*10*). This was recrystallized four times without significant change in its specific radioactivity. The melting point of the recrystallized derivative was 189° to 190°C. Eighty-five percent of the radioactivity in column fraction 3 was established to reside in retinal by this procedure, after an incubation in which 29 percent of the radioactivity was recovered in fraction 3 (retinal) after column chromatography.

Calculations based on the stoichiometry of our results indicate that most of the reaction-product retinal must have arisen by central cleavage of the substrate β-carotene into two molecules of retinal. Thus, in one experiment three incubations were conducted without enzyme or with inactivated enzyme, and four incubations were conducted simultaneously with active enzyme. The same amount of substrate was added to each of the seven incubation flasks. After incubation, 1029 ± 13 count/min (*11*) were recovered in column fraction 1 (carotene) and 55 ± 9 count/min in fraction 3, from the three flasks without active enzyme. After incubation with active enzyme, 535 ± 28 count/min were recovered in fraction 1 and 460 ± 20 count/min in fraction 3. These results are not consistent with the possibility that only one molecule of retinal is formed from one molecule of β-carotene.

During the biosynthesis of vitamin A, therefore, β-carotene presumably reacts with molecular oxygen, and this reaction is followed by the cleavage of the central double bond to form two molecules of retinal. Retinal is then reduced to retinol, which is subsequently esterified, mainly with palmitic acid (*4, 5*). Glover, Goodwin, and Morton have demonstrated the ready reduction of retinal to retinol by the intestinal wall in vivo (*12*), and experiments in our laboratory have demonstrated that this reaction is mediated by a soluble protein, in the intestinal mucosal homogenate, which requires the reduced form of nicotinamide adenine dinucleotide as cofactor. The newly formed retinyl ester is then mainly incorporated into lymph chylomicrons (*4, 5*) and is subsequently transported by way of the intestinal lymphatics, to eventually enter the vascular compartment.

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Trend Curves of the Rate of Species Description in Zoology

Abstract. *Trend charts of approximately 0.4 percent of the animal kingdom indicate that the describing and naming of species, "alpha taxonomy," is far from completed.*

Abelson (*1*) expresses a widely held opinion in saying that "work on the descriptive features of gross morphology has largely been completed, and the rate of discovery and description of new species has slackened." Blackwelder (*2*), however, says that "it must be recognized that the job of making known the animals of the earth is so far from finished that we don't yet know even the general pattern of relative abundance of all the major groups." The conflict here calls for some kind of factual background that so far seems to be lacking.

A few years ago I started to compile numerical data concerning the rate of description of species in some insect groups. The results were so encourag-

ing and illuminating that I extended the effort.

Biologists make much use of statistics in many branches of their science, but one division of statistics, that in which time is a factor, has been almost entirely neglected. Personnel in industry, commerce, sociology, and other fields have made much use of "trend curves" to forecast conditions or to gain an insight into the relative position in time of a present condition. An amusing account of the ways by which this is done, but one containing a large amount of wry truth, is to be found in an issue of the magazine *Analog Science Fact and Fiction* (*3*). For the biologist or taxonomist who is only secondarily interested in statistics, two inexpensive but highly commendable paperbacks may be cited (*4*), both of which contain bibliographies.

If we accept as one premise the existence of a finite and practically fixed number of species on the earth, and for a second premise that we have been gradually approaching a state in which all the species would be known, at least to the degree at which a specific name could be applied, then it should be possible to present graphically the rate at which this knowledge has been gained from the time it was systematized (1758) until the present. The shape of a curve thus obtained should permit some kind of rough prediction as to our present position on the road from ignorance to relatively complete knowledge.

By using recent lists and revisions (unfortunately, all too few are available) with up-dating from *Zoological Record* (*5*) and other sources, and by plotting accumulated numbers of species on ordinates and years by decades from 1758 (1760) to the present on abscissas, a basis for drawing a curve was obtained. In Fig. 1 results of such plotting of several animal groups are shown. When the curve is sigmoid, one may infer that the naming-describing process is relatively complete. Many of the curves describe the taxonomic state of insects because I am an entomologist and the literature of insects is most familiar and available to me. Incidentally, insects comprise by far the largest of all animal groups.

In compiling the data, I counted only species at present considered valid, and I considered no infraspecific data except for those in Fig. 1*a*. When only a nontypical subspecies occurred in a regional compilation, I used the date

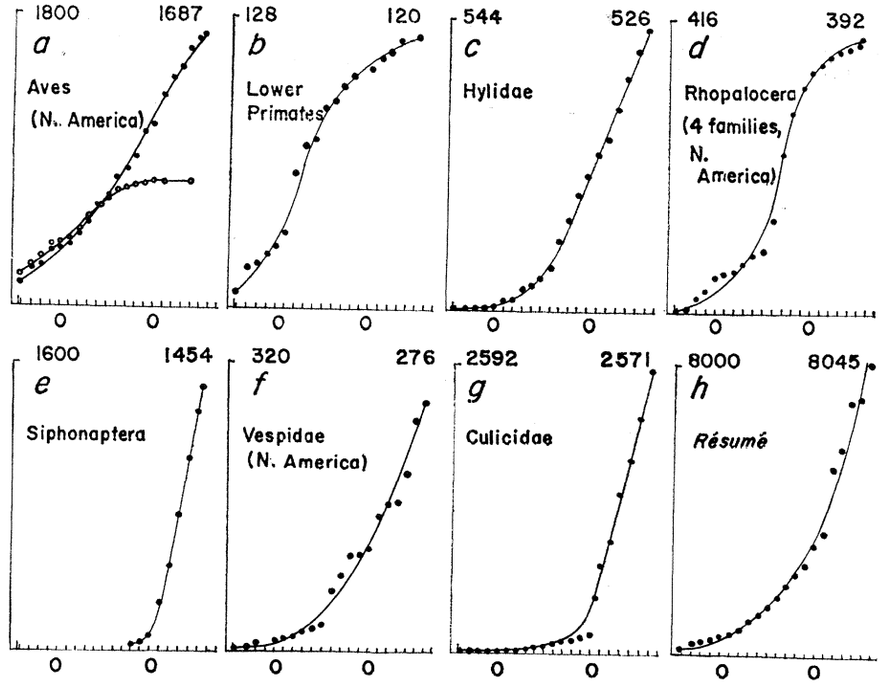

Fig. 1. Trend curves of rate of species description. The numbers of species are given at the upper end of each curve and the number of species scaled on the ordinates are indicated at the upper right and left edges of each figure. The bottom line (decades) begins at the left with 1758, then 1760 and succeeding decades, with zeroes at 1800 and 1900. *a*, Open circles represent species; closed circles, subspecies. *e*, 1800–1954; includes subspecies (after Smit, *6*).

of the typical subspecies; for example, only *Vespa crabro germana* Christ is found in North America, but I used the date of *V. crabro* Linnaeus 1758. I placed dots at accumulated numbers of presently valid species at the end of each decade of years, then drew a curve from the first dot to the last in such a way as to connect the maximum number of dots and to leave on each side of the curve an equal number of dots. Where no dot was placed, no species was added in that decade.

In several of the charts the dots are fairly scattered. This results from such fortuities as the number of workers, time of publication, exploration of new regions, new techniques (especially use of genitalic characters); when the work was conducted by large numbers of workers at frequent intervals, without sudden changes in technique or new areas of exploration, the curve follows the dots quite closely (see Fig. 1, *a–c* and *e*).

From purely theoretical considerations, a curve that is rising steeply cannot suddenly level off, since the possibility that during a rapid advance a stage would be suddenly reached at which the last few, rare, out-of-the-way, obscure, or cryptic species would be made known is infinitesimal. Therefore, we must presume that when the curve rises sharply, a considerable number of species remain unknown and that some kind of balance exists between the slow accumulation of data and development of technique and the final gathering of straggler species and ultimate refinement of technique and concepts.

On the other hand, if the curve appears to be leveling off, we have no assurance that we are approaching the stage at which all species are known. A time of lagging taxonomic work, especially in a small group with few workers, may easily produce such a condition. Then, with a new and assiduous worker in the field, sudden recognition of economic importance of one or more species in the group, or the use of a new technique, the curve will shoot upward. Consequently, the chance that the chart will show a finished group is much less than that the group is far from completely known.

The only similar chart I have been able to find is one by Smit (*6*), the data from which are presented as Fig. 1*e*, drawn in accordance with my design.

The first three figures deal with vertebrate groups. In Fig. 1*a* (*7*), it is ap-

parent that the species of North American birds are well known, but that much work is still necessary in regard to subspecies. The congruence of curve with dots here is very good. It should be noted that with such a beloved group, we started in 1758 with a fair amount of knowledge. Even in the lower primates [Fig. 1*b* (*8*)], we note that a few species must still be unknown. The tree-frogs, or Hylidae [Fig. 1c (*9*)], yield a curve with surprising congruence of line and dots and one that indicates a large number of unknown species. The discovery of the importance of the Culicidae, or mosquitoes (Fig. 1*g*), as carriers of disease at the end of the last century together with the use of genitalia in identifying them brought a sharp rise in the number of species. Probably only the curves in Fig. 1*a* (species), 1*b*, and 1*d* really indicate that completion is near. It should also be noted that there is little correlation between the economic importance of a group and the smoothness of its curve; the curve for tree-frogs (Fig. 1*c*), which are of practically no economic importance, is much smoother than those for butterflies (Fig. 1*d*) and Vespid wasps (Fig. 1*f*), many of which are of economic importance.

The last chart (Fig. 1*h*) is a composite of all the preceding groups plus several not figured, representing a combined total of 8045 species. This is but a small sample of the Animalia, possibly only about 0.4 percent, but it should provide us with an indication of the long road still ahead.

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Insect Mating Behavior: Endocrine Control of a Chemical Communication System

Abstract. *Experiments on several species of moths and cockroaches indicate that the production of sex pheromone (a male attractant) in virgin females is under endocrine control in some species but not in others. The presence or absence of endocrine control over pheromone production may be correlated with the type of life cycle exhibited.*

Virgin females of many species of insect produce sex pheromones which attract males and initiate male precopulatory behavior (*1*). A relatively simple chemical communication system of this type underlies the mating behavior of the cockroach, *Byrsotria fumigata* (Guérin) (*2*). The mating behavior of this cockroach is under the control of the endocrine system—specifically, the production of the female sex pheromone is under the control of the corpora allata (*3*, *4*). Results previously reported may be summarized as follows: (i) adult females ordinarily fail to produce sex pheromone if their corpora allata have been removed shortly after the imaginal molt; (ii) there is a high correlation between the production of sex pheromone and successful mating (that is, insemination); and (iii) the implantation of corpora allata into previously allatectomized females can induce pheromone production. Subsequently, mating tests in which allatectomized females (artificially coated with sex pheromone to enhance their attractiveness) were exposed to males have shown that pheromone production is the only aspect of the female's sexual behavior affected by the removal of these endocrine organs (*5*).

These data suggest that mating behavior may be similarly regulated in other insects. In the light of this possibility, the results of allatectomy will be examined in three other insect species; one cockroach, *Pycnoscelus surinamensis* (L.), and two moths; a saturniid, *Antheraea pernyi* Guérin, and a pyralid, *Galleria mellonella* (L.).

There are two different strains of the cockroach *P. surinamensis*; one strain is bisexual and the other parthenogenetic (*6*). Roth (*7*) discovered that females of the parthenogenetic strain (as well as those of the bisexual strain) produce a sex pheromone which stimulates the courtship behavior of males of the bisexual strain, when assayed by the filter-paper technique (*3*). In the present study, the effect of allatectomy on the production of pheromone by virgin females differed in the two strains (Table 1). In the bisexual strain, removal of the corpora allata less than 24 hours after the imaginal molt resulted in a failure of sex pheromone production in most experimental animals. Thus, the corpora allata appear to control the production of sex pheromone in animals of the bisexual strain as they do in *B. fumigata*. By contrast, removal of the corpora allata just after the imaginal molt had no effect on pheromone production in parthenogenetic females. Pheromone production occurred normally in the adult even in females allatectomized during the last nymphal instar. Thus, in the parthenogenetic strain the corpora allata seem to have lost their ability to control pheromone production. Further evidence for this conclusion may be derived from observations on females carrying egg cases. The corpora allata control oocyte maturation in females of both strains and the activity of these glands is inhibited while an egg case is being carried (*8*). Females of the bisexual strain do not produce pheromone while carrying egg cases, which is as would be expected from the known inhibition of the corpora allata at this time. By contrast, in most parthenogenetic females tested while carrying egg cases, pheromone production persisted, thus indicating its independence from control by the corpora allata. Hence, in this case in which there can no longer be any selective advantage in being able to signal to the male the female's ability to mate, the endocrine control over the necessary communication system appears to have been lost (*9*).

These results lead to the hypothesis that the regulation of mating behavior by means of endocrine control of sex pheromone production may not be a widespread phenomenon in insects. Rather, it may be expected to occur only in those instances in which selection pressures favor the evolution of such a mechanism. This would be the case in insects such as cockroaches which are long-lived in the adult stage and which have repeated reproductive cycles in which there are periods during which successful mating is not possible. In insects which are short-lived as adults, which lay eggs and die within a few days, the female must attract a male within a very