at the Indian level were grouped in indeterminate category. A large number of Indian species falls under indeterminate category, since we do not have any solid quantitative data. This is a sad and inadequate situation from the scientific point of view. In India, a lot of work needs to be done on identification, mapping and distribution of indigenous species for developing strategies for their conservation. The detailed information further strengthens the conservation of threatened plants.

In any attempt to develop high priority 'Conservation area matrix', endemic flora and fauna take precedence over other 'wide' species, as endemics once lost, is a loss of biodiversity forever[3]. Many species of our endemic flora are known only from type localities and some of them have not been collected after the type collections. Of the 47 species known from type collection in the Eastern Ghats, only three have found place in the *Red Data Book*[4]. It is now for the botanists in different parts of the world to critically examine the entries in the *Red Data Book* and IUCN Red Lists and prepare a list of threatened plants. In order to understand the rarity of species, it is necessary to study the biology of species and environmental factors affecting the species. This will draw attention to programmes of conservation of the threatened species. We need to raise the levels of our perception and evaluation.

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Looking beyond number of publications and journal impact factor

According to the current scenario, the criteria for any level of candidacy evaluation among scientists seem to be centred first on the impact factor of journals (JIF)[1] and on number of publications (Σ_{Pub}). While doing so, we should not overlook the possibility and probability of attaining both criteria, as the JIF and the temporal pace to accumulate Σ_{Pub} vary across subjects and/or fields of research. Only the glaring high-impact journals like *Nature*, *Science* and *PNAS* allow space for wide- and multi-disciplinary research articles. Obviously, these journals are open to a wide array of researchers dealing with science, and those publishing in these journals, on any topic, get the same impact factor.

However, as a matter of fact, researchers use to publish frequently in subject-specialized journals. Here we must note that the average/median JIF of each subject and then its relevant sub-fields markedly vary (source – Journal Citation Reports 2006, ISI Web of Science). Take for instance the two extreme fields in biology, in terms of median and aggregate impact factor; cell biology (the high end) and entomology (the low end). Accordingly, if we compare and/or analyse the researchers in the aforementioned fields, we would definitely find notable differences in average/aggregate impact factor of, say for instance, ten publications of a cell biologist and ten publications of an entomologist. This is at least widely known among scientists and academic visionaries.

But what is still seldom noticed/considered is the intra-field variation in impact factors. Let us take ecology as an example. This major discipline has several sub-disciplines, some of which are inter *inter-disciplinary* (e.g. physiological ecology, chemical ecology and molecular ecology) and others are inter *intra-disciplinary* (e.g. plant ecology, animal ecology, community ecology, evolutionary ecology, ecosystem ecology and conservation ecology). Conceivably, we have many sub-fields branching out from each and every sub-disciplinary tree. So, scientists working in the various subjects indicated above, vary remarkably in their pace for quantity (Σ_{Pub}) and quality (average impact factor). Consequently, the number of citations per paper would vary across the subjects indicated above and it also entails several other factors[2–4].

Even if we explore within a level playing field, the histogram of most early-career scientists' publications will have a peak near average JIF of their respective subject. Furthermore, it is not uncommon for researchers to have significant/groundbreaking contributions in journals with JIF less than their (other) usual/minor contributions in journals that may have relatively high JIF. These are possible because of various reasons: (1) compatibility between the contributions and scope of the journals, (2) as groundbreaking contributions arise incidentally, one might wish to publish it quickly with enthusiasm, even in a low- or medium-impact journal, before acquiring enough data to document it in high-impact journals.

So, this gives rise to a complex scenario on how to evaluate publications of different candidates, peaking near average JIF. The solution is to carefully consider the significant contributions (through publications as well as other contributions that lead to the development of new projects in the laboratory) made by a candidate, rather than just looking at JIF and Σ_{Pub}. This is an important criterion that is being often overlooked. Though mean number of genuine citations[3,5] (i.e. excluding attacking and self-citations) per paper or *h*-index[5] is being considered a key tool, it is not applicable for early career scientists[4].

Only in the education and research centres of excellence, do the juries have

a keen eye to the complex scenario, and they make reasonable evaluation while recruiting new faculty. Whereas, in most other places, the candidacy evaluation by concerned authorities seems to be a mosaic in their peering of the number of publications and JIF within a major discipline, e.g. cell biology, neurobiology, ecology, evolution, etc. Perhaps, such mosaic evaluation may (partially) exist even at higher levels while rewarding research excellence within a major discipline[6]. At the outset, the evaluation of science is complex and it begs an eagle's eye-view approach, as suggested by Gadagkar[1] and Balaram[4].

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Recombineering: potential for generation of live attenuated bacterial strains

Infectious diseases remain one of the largest killers of mankind. The development and availability of a number of vaccines against the viral and bacterial pathogens has reduced mortality a great deal. Eradication of smallpox and near eradication of poliomyelitis had only been possible due to the availability of good vaccines and effective immunization. Bacterial diseases such as tetanus and pertussis could be controlled due to active immunization. Among the different types of vaccines, live attenuated remain one of the most effective vaccines due to their ability to elicit both humoral and cellular immune response[1].

Live attenuated bacterial strains have traditionally been generated by chemical mutagenesis or serial passage through non-permissive host. The early approaches lacked specificity and were replaced by techniques of reverse genetics. The method required better understanding of pathogenic mechanisms and genome sequencing; and targeted inactivation or deletion of the virulence-associated genes. We know that the bacterial pathogens owe their disease-causing ability to the presence of virulence traits encoded by the various genes present either on chromosomes or plasmids. Typically, the techniques of reverse genetics or classical allelic exchange system involved engineering recombinant-replication defective plasmids (suicide vectors) to contain long stretches of homologous DNA sequences flanking the genes to be deleted. Construction of such recombinant plasmids requires extensive *in vitro* manipulations. Hence, the method is laborious and time-consuming. However, it had been extremely useful in generating mutants of various bacterial species.

Since the late 1990s, new *in vivo* technologies of homologous recombination have emerged that have greatly simplified the process of target gene deletion. Among these, one of the most promising techniques is 'recombineering', which utilizes the lambda recombination proteins Bet, Exo and Gam, encoded by 'red' genes and linear ds or ss DNA with short homologies to the target gene[2,3]. The Gam protein prevents degradation of the linear ds DNA from recBCD or SbcCD of host bacteria, whereas Exo and Bet help in the homologous recombination of the linear substrate. The linear ds DNA can easily be generated by PCR and requires as little as 50 nt homology to the target gene. The red genes of bacteriophage lambda can be cloned on the plasmid under control of heterologous, inducible promoter and their expression can be regulated by temperature or arabinose. Recombineering has several advantages over the classical allelic exchange system, e.g. greater ease of the technique, increased recombination efficiency, smaller region of homology and most importantly, the technique requires less time.

Recombineering has been used to modify the genomes of *Escherichia coli*, *Shigella*, *Yersinia*, *Salmonella*, and enteropathogenic and enterohaemorrhagic *E. coli*[3–6]. These studies have used homologies of 35–500 nt to engineer the target gene deletion and generate a candidate prototypic live, attenuated vaccine strain. In our experience, we have found that homologies of about 300–400 nt were required to make a *phoP–phoQ* null mutant of *Yersinia pseudotuberculosis*[7]. The mutant with defined genetic lesions can be made targeting either the virulence or the regulator gene(s). Many of the live, attenuated prototypic vaccine strains generated by recombineering look to be promising for their vaccine potential[4,5]. With the option of gene manipulation even in plasmids, as may be applicable in *Yersinia pestis*, recombineering can be a promising technology for producing the prototypic live attenuated bacterial vaccine strains in future.

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