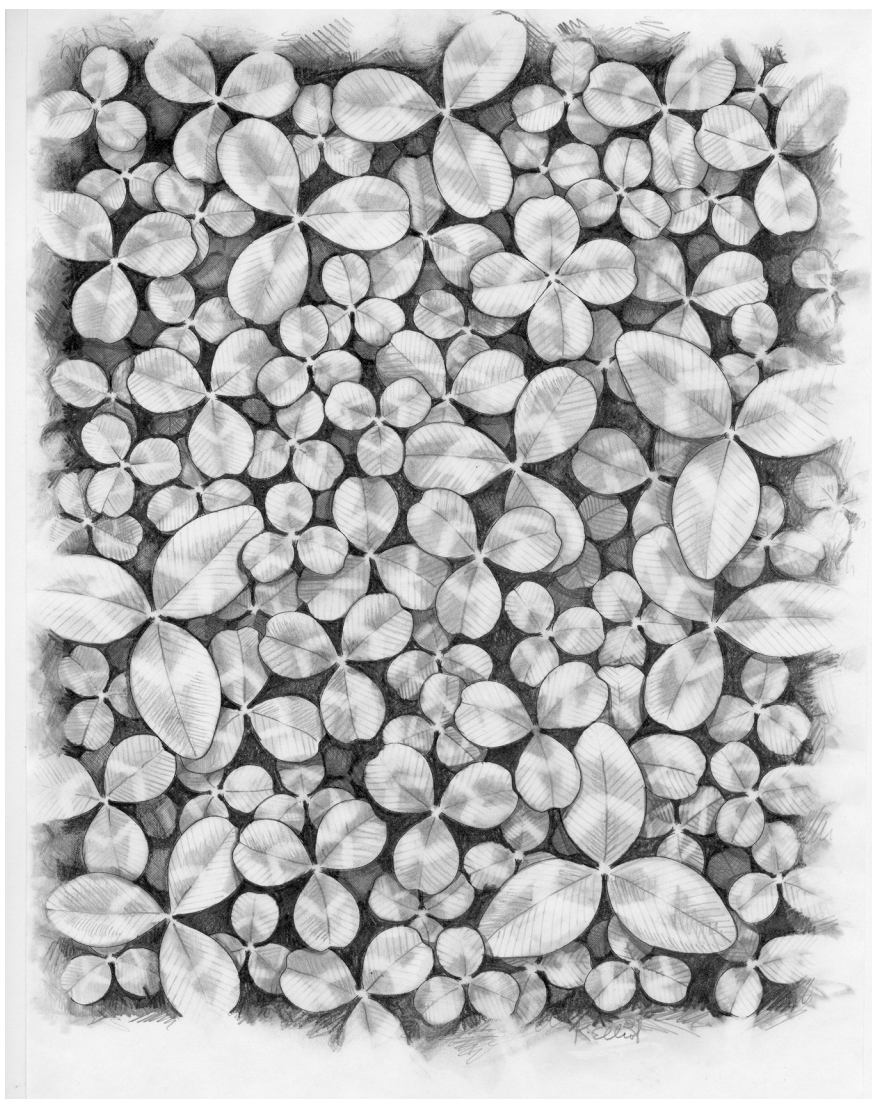




Genetic Variation

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Diversity is a way of coping with the possible.

François Jacob, 1982 p. 66.

*Genomic components have significance only
in terms of phenotypic expression.*

Steven M. Stanley, 1979 p. 56.

2.1 Introduction

What processes account for the remarkable variation in form and function that we see in the living world and how might they be similar or different between microorganisms and macroorganisms? Because natural variation provides the raw material on which evolution acts, this chapter sets the stage for later comparisons in the book.

Here we review briefly the generation, maintenance, and transmission of genetic variation in micro- and macroorganisms. Microbes generally have plastic, dynamic genomes and all the capability of generating genetic variability that do macroorganisms, though adaptive evolution proceeds rather differently in the two groups. We discuss clones and what constitutes a ‘genetic individual’ in various taxa and show that the concept is more-or-less abstract, with major evolutionary implications. The important role that the cellular and external environments plays in reciprocal interactions with the genome and its phenotypic manifestation is developed in ►Chap. 7. The major kinds of change that affect the central information (DNA) repository for cells are mutations; rearrangements, and recombinations; and epigenetic and other gene expression controls. Changes in sequence information typically manifest themselves on a scale of multiple organism generations, whereas at least some epigenetic and related gene regulatory changes, such as prions, can be gained or lost quickly and at higher frequencies than DNA mutations, i.e., within the cycle of a single cell or involving a few cell generations (►Chap. 7 and Shapiro 2005; Jarosz et al. 2014 a, b).

Arguably the biggest revolution in genetics of the past half-century has been the shift in analytical focus from gene to genome. The term ‘genome’ has been used in many contexts; Shapiro (2002, p. 112) defines it as “all the DNA sequence information of a particular cell, organism, or species.” Along with this paradigm change is recognition that the latter is fluid and internally dynamic, rather than static and subject to change only through background random point mutation. A computer storage system metaphor for genome function is described by Shapiro (2002, 2011), summarized as follows: The genome contains many categories of information, each associated with a certain sequence code. Among these are the well-known coding sequence for RNA and protein; sites for initiation and termination of transcription; sites for covalent modification of DNA; and binding sites for the spatial organization of the genome. So the genome is in effect formatted for various functions it carries out in conjunction with other cellular systems designed to read, replicate, transmit, package, or reorganize the DNA sequence information. Such formatting is analogous to the formatting of computer programs wherein various generic signals recognize particular files regardless of their specific content. Among species there are differences in how control systems operate. Bacteria handle regulatory decisions somewhat differently from eukaryotes, just as different computer systems recognize different codes. New data and new programs are written when the genome is restructured by natural genetic engineering that can occur in many ways (►Tables 2.1 and 2.2).

Table 2.1 Changing concepts and metaphors related to genetic information and variation (from Shapiro 2002 with minor rewording and abbreviation)

Process or concept	Classical genetics era	Molecular genetics era
Hereditary theory	Genes as the unit	Genomes as interactive systems
Genome organization	Beads on a string	Computer operating system
Sources of inherited novelty	Localized mutations due to base changes; one gene affected at a time	Epigenetic modifications and rearrangement of genomic components due to internal genetic engineering
Evolutionary processes	Natural selection acting on independent background random mutations; cells passive	Non-random, genome-wide rearrangements; cells active in restructuring genome

Table 2.2 Some mechanisms and consequences of DNA restructuring (natural genetic engineering; abbreviated and slightly revised from Shapiro 2002)

DNA reorganization process	DNA rearrangements involved (examples)
Homologous recombination	Reciprocal exchange; unequal crossing-over; gene conversion
Site-specific recombination	Insertion, deletion, or inversion of DNA carrying specific sites
Site-specific DNA cleavage	Direct localized gene conversion by homologous recombination; create substrates for gene fusions
Nonhomologous end-joining systems	Precise and imprecise joining of broken DNA ends; genetic fusions; localized hypermutation
DNA transposition	Self-insertion; transposons carry signals for transcriptional control, RNA splicing and DNA bending; amplifications
Retroviruses and other terminally repeated retrotransposons	Self-insertion and amplification; carry signals for transcriptional control, RNA splicing and chromatin formatting; mobilization of sequences acquired from other cellular RNAs

2.2 Mechanisms

2.2.1 Mutation

Mutations are the ultimate source of most variation in all organisms. By definition (e.g., Barton et al. 2007), they consist of changes in the genetic message (either at the level of the gene or the chromosome) that are heritable and, implicitly, detectable. Mutation generates the variation; recombination amplifies this by mixing it, in essence by repackaging it, not by creating brand new packages. We note in passing that molecular biologists frequently speak of inherent mutation rates (usually referring to replacement rates of one DNA base by another, as may be read, for example, from a genomic sequence), whether or not there is a change in the wild-type phenotype. This fundamental level of mutation may go undetected if the protein is not functionally altered, if two or more mutations compensate for each other

giving a pseudo wild-type, or if another gene product takes over the function of the altered protein. Thus, there are really three mutation rates—the inherent rate of uncorrected change in DNA sequences; the rate at which the mutants survive; and the rate at which survivors are detected in a population by virtue of their phenotypic differences. Although there are many types of mutation, fundamentally most involve errors introduced into the genome. These may occur spontaneously or be induced by endogenous or exogenous mutagens, typically occurring during replication; and errors in recombination, repair, or in large-scale chromosomal rearrangements (Alberts et al. 2015; Griffiths et al. 2015). To these long-established sources of failure in cellular machinery must now be added the more recently recognized category of genomic change caused by mobile genetic elements such as transposons (below).

Mutations are mechanically inevitable—damage to DNA occurs and copying, proof-reading, and segregating mechanisms are not and cannot be perfect. For this reason Williams (1966, p. 12) has argued that mutations are not adaptive, i.e., they should not be considered to be a means for ensuring evolutionary plasticity. That they still occur is largely in spite of natural selection rather than because of it (Williams 1966, p. 139); However, this does not mean that there cannot be selection for a particular mutation *rate* and there are clear differences in rates among species (below). Overall, because many of these alterations are deleterious, there has been strong selection pressure (reduced reproductive fitness) over millennia to reduce rates in organisms. Incremental benefit in increased fidelity below a certain point may be offset by greater physiological costs in correction (Kimura 1967; Drake 1991). It has also been argued (Lynch 2010) that the lower limit is set by genetic drift. Regardless, there is some value in having background genetic ‘noise’ (Drake 1974; Drake et al. 1998). This is illustrated by the essential function of mutation (somatic hypermutation) in antibody genes of the B lymphocyte cells, which contributes to antibody diversity in vertebrates (French et al. 1989). Analogous genetic rearrangements provide the operon structure needed for expression of *nif* genes in a nitrogen-fixing cyanobacterium, *Anabaena* (Golden et al. 1985). The terminal step in heterocyst differentiation involves excision of DNA in response to an environmentally triggered, site-specific DNA recombinase (Haselkorn et al. 1987). Thus, much the same rearrangement process is used in a prokaryote and an advanced eukaryote.

With the mechanistic characterization of transposable elements in both prokaryotes and eukaryotes (for an overview, see Griffiths et al. 2015), the distinction at the molecular level between mutation and recombination is often one of semantics. Classification schemes are more-or-less subjective because the degree of genetic change occurs as a continuum. For instance, where relatively large segments of DNA (hundreds or thousands of nucleotides) are rearranged, such as by inversion or exchange between chromosomes, the event is often called mutation by molecular biologists (Chaps. 9 and 11 in Watson et al. 2008). Yet it falls within the domain of recombination (in the sense of mixing of nucleotides) as defined by eukaryote geneticists. For example, site-specific recombination (see Recombination, later) is a mechanism that can lead to mutation. Probably the best-known form of recombination is the even exchange of sequences between aligned, homologous chromosomes at meiosis. However, recombination can occur between any two regions of sufficient DNA similarity on the same chromosome or between paired chromosomes, an event known as ectopic (nonallelic) recombination. This potentially leads to mutation due, for example, to deletions or inversions (in the case of intrachromosomal recombination) or, if two chromosomes are involved, to unequal crossing-over where one offspring receives too many copies of the sequence and the other too few (Chap. 12, Barton et al. 2007).

Before proceeding into the details, some important general points about mutations need to be made. First, they occur in somatic as well as in germline cells, the implications of which will be discussed in ►Sect. 2.5 (see Shendure and Akey 2015). Suffice it to say here that, with few exceptions (such as among the somatic cell lines that produce antibodies), high rates must be avoided in *both* lineages. The implications of mutation in somatic cells tend to be overlooked because of the focus by evolutionary biologists on the germline.

Second, the impact of a mutation will depend on the environment, broadly construed (►Chap. 7). A mutation deleterious in one set of circumstances could be beneficial in another. Also, as discussed later, while mutation rates can vary with environmental conditions, the environment does not induce mutations that are specifically adaptive; i.e., adaptively directed mutation does not occur (Barton et al. 2007; Futuyma 2009). Current evolutionary thinking is that mutation and selection are separate processes. It was well established for bacteria by Luria and Debruck in the 1940s, confirmed by the Lederbergs in the 1950s, and reconfirmed with elaborations since then, that mutations conferring resistance to phage or antibiotics happen both before and after the selective agent is applied and are random with respect to their adaptive value (Barton et al. 2007). (For commentary on the controversy that some mutations in bacteria are adaptive, allegedly being ‘directed’ by the environment, see Sniegowski and Lenski 1995; Foster 2000; Sniegowski 2005.)

Third, the impact of a mutation can depend on the stage of development of an organism. Mutations occurring in early ontogeny, for example, in animals at the blastula stage, are much more likely to be lethal because of their far-reaching impact on subsequent differentiation. In contrast, those occurring later may affect relatively superficial properties such as eye color (Bonner 1965, pp. 123–128; 1988, p. 168). As Bonner notes, it follows from this that any mutation at *any* stage in the life of a unicellular organism is more likely to be lethal than would a similar mutation during most of the life of a macroorganism.

Extent of mutations If we categorize mutations by the degree of the base changes involved, the first kind consists of simple base position (typically called point) substitutions such as a C to a T in the nucleotide sequence, or by addition or deletion of a single base (Graur and Li 2000; Griffiths et al. 2015). Because of the degeneracy feature of the genetic code (‘wobble’ in the third codon position), often there is no change in the amino acid sequence (‘synonymous’ or ‘silent’ mutation). Mutations may also be silent if a related amino acid is inserted (**missense conservative** mutation) or if the affected region of the gene product is unimportant. If the altered codon specifies a different amino acid, the change is a **missense nonconservative** mutation. This is manifested either as an altered but functional (‘leaky’) protein product or as a defective protein. The latter category is very common, as in sickle cell anemia, Tay-Sachs disease, and phenylketonuria. One form of retinitis pigmentosa is apparently caused by a C to A transversion in codon 23, corresponding to a proline to histidine substitution (Dryja et al. 1990). Finally, if translation is terminated (**nonsense** mutation), a shortened and likely defective protein results.

It should be emphasized that point and other small changes in the **noncoding** regions of a gene, such as in regulatory sequences, can potentially be very significant in evolutionary terms. For example, such changes may create or interrupt a binding site thereby changing or even obliterating the expression of the gene. In other words, alteration in gene regulation, accomplished in multiple ways, has arguably had an even greater impact than actual mutation in the polypeptide coding regions of those same genes (see later section and ►Chap. 7; also Doebley and Lukens 1998; Carroll et al. 2005).

Second in extent of nucleotides involved are small insertions or deletion (**indel**) mutations involving one or a few base pairs. When this happens within regions coding for polypeptides, the mutations are called **frameshift** if the reading frame of the codons is shifted out of phase (Barton et al. 2007; Alberts et al. 2015). This can lead to premature termination or obliteration of a stop codon, so a frameshift mutation may result in a truncated, possibly unstable protein, or it may generate a completely nonfunctional product, depending on its position in the structural gene. In general, the foregoing changes, except deletions, are revertible to wild-type. Reversion of deletions depends on the nature of the deletion and the encoded gene product.

The third category of mutation comprises the potentially large-scale chromosomal changes such as bigger deletions, inversions, duplications, and insertions (transpositions) that intergrade with recombination, discussed later. Thus, the size or number of genes in chromosomes may be increased or decreased; genes may change in location (by inversion or translocation); and there may be wholesale changes in the number of chromosomes. Where a deletion is large enough to remove or disrupt a gene coding for a critical enzyme, the result is likely to be death of the cell or organism. At the other extreme, while polyploidy (more than two chromosome sets) is generally considered to be an abnormal condition, it appears to be well tolerated by some organisms, especially plants. Here, it is estimated to have resulted in 7% of the speciation events in ferns and 2–4% in angiosperms (Otto and Whitton 2000), as well as in the associated evolution of plant gene families and structural complexity (De Bodt et al. 2005). Most such polyploidizations are followed by gene deletions over time resulting in eventual diploidy. Plants, however, again are distinctive in being able to tolerate imperfectly matched chromosomes that result from loss or rearrangement (Walbot and Cullis 1983). Stanley (1979) has emphasized the role of chromosomal rearrangement together with gene regulation in ‘quantum speciation’.

Gene expansion, contraction, duplication, or loss may contribute significantly to the evolution of complexity, functional diversity, and adaptation to the environment over evolutionary or contemporary time (see ►Chap. 4 and also: Verstrepen et al. 2005; Hittinger and Carroll 2007; Pr nting and Andersson 2011; Andersson et al. 2015). A classic example is the evolution of the globin gene family in multicellular animals and how duplication was instrumental in creating new proteins (Hoffmann et al. 2012; Alberts et al. 2015). Analogously, the evolutionary expansion of enzyme families in plants associated with specialized metabolism for which plants are famous is attributable to various kinds of gene duplication events (Moore and Purugganan 2005). In contrast, genes can be inactivated by insertion of mobile DNA elements into the chromosome and subsequently eroded or lost. These insertions may be fragments (generally 1,000–2,000 nucleotides) that do not code for any characters beyond those needed for their own transposition (simple transposon or insertion sequence; discussed later in Recombination section). Alternatively, they may be longer elements (complex transposon or transposable element) that code for a product such as antibiotic resistance in bacteria. The rates of gene loss and gain are particularly dynamic in prokaryotes due in large part to a process of horizontal or lateral gene transfer (discussed later under Sex and Adaptive Evolution in Prokaryotes), offset by inactivation and deletion (Mira et al. 2001; Lerat et al. 2005). Genome plasticity is also notable in plant (Ibarra-Laclette et al. 2013) and fungal (Raffaele and Kamoun 2012) evolution.

Rates As alluded to above, mutation rates can be estimated by recording spontaneous phenotypic changes over time in a population of organisms in nature (animals or plants) or in the laboratory (tissue culture or microbial culture). Because the frequencies of most muta-

Table 2.3 Mutation rates per nucleotide site ($\times 10^{-9}$) for selected species and tissues (abbreviated from Lynch 2010)

Species	Tissue	Cell divisions per generation	Mutation rates	
			Per generation	Per cell division
<i>Homo sapiens</i>	Germline	216	12.85	0.06
	Retina	55	54.45	0.99
	Intestinal epithelium	600	162.00	0.27
<i>Mus musculus</i>	Male germline	39	38.00	0.97
	Liver		237.88	
<i>Rattus norvegicus</i>	Prostate		448.90	
<i>Drosophila melanogaster</i>	Germline	36	4.65	0.13
	Whole body		380.92	
<i>Caenorhabditis elegans</i>	Germline	9	5.60	0.62
<i>Arabidopsis thaliana</i>	Germline	40	6.50	0.16
<i>Saccharomyces cerevisiae</i>		1	0.33	0.33
<i>Escherichia coli</i>		1	0.26	0.26

tions are very low in natural populations and can only be detected in large sample sizes, it is common to examine specific proteins electrophoretically or DNA sequences directly (e.g., in restriction fragment length polymorphisms, or as single nucleotide variants on a genome-wide basis). The mutations tallied and mutational rates published typically pertain to single nucleotide substitutions and, in some cases, indels or copy number variants (e.g., Lynch 2010; Shendure and Akey 2015), but rarely thus far on large structural (>20 bp) variants (Campbell and Eichler 2013; Kloosterman et al. 2015).

Rates are expressed in numerous ways, e.g., on the basis of per base pair per cell replication; per genome per replication; or per genome per sexual generation (unless otherwise stated, typically on a haploid basis). For an example of such data see **Table 2.3** and Lynch (2010). Different eukaryotes (or even sexes within a species) have different numbers of cell divisions involved in gamete production per sexual generation. In humans there are about 30 germline cell divisions in females, but more than 100 for spermatogenesis in males (Crow 2000; Barton et al. 2007), which thus have a higher mutation rate on a generational basis. Higher eukaryotes have a genome up to several orders of magnitude larger than viruses or prokaryotes, but most of this is in introns and inter-genic regions where most mutations are neutral rather than functional genes. Thus, rates for macroorganisms are often expressed as **per effective genome** (i.e., excluding the genomic regions where most mutations are neutral) per replication (cell division) or per sexual generation (Drake et al. 1998). There are further complications or caveats with eukaryotes, among them age- or sex-related

effects alluded to above, as well as substantially higher rates in somatic versus germline cells, discussed later. Nevertheless, as a generality, when expressed as number of mutations per base pair per replication, there are appreciably higher rates for smaller than larger genomes. When expressed on a per genome basis, there is less variation across genome sizes or species. Rates in microbes are typically about 1/300 per genome per replication (versus about 1 per genome per replication event in the lytic RNA viruses; Drake et al. 1998). **For the higher macroorganisms, on an effective genome basis, rates per sexual generation are in the range of 0.1–100, but on a cell division basis per effective genome are approximately comparable to the 1/300 for microorganisms** (Drake et al. 1998; Hershberg 2015). The remarkable comparability across taxa remains to this day a source of animated debate as to, for example, whether it reflects an inherent rate of change or a common level of error correction. The implication of various determinants on the ultimate mutation rate, including factors such as sexual versus asexual reproduction and population size, has been discussed at length (see e.g., Drake et al. 1998; Lynch 2010; Sung et al. 2012).

Beyond the differences noted above, the incidence of mutation in a local sense varies with numerous other factors. Among these are the specific genomic region (so-called ‘hotspots’ vs. ‘coldspots,’ in part affected by degree of DNA superhelicity; see Foster et al. 2013), organelle (nucleus vs. mitochondrion), and type of base (AT-biased rather than GC). Thus, whereas mutation is generally described as a random phenomenon, where it occurs in the genome is far from random. The consequences of mutation are inextricably linked to life cycle (►Chap. 6). For instance, mutations are masked by diploidy and even more so by polyploidy. In relatively simple organisms (such as algae with a prominent haploid gametophyte phase, see ►Chap. 4) the individual experiences selection mostly in the haploid stage of the life cycle. Under such circumstances mutations will not be masked and there will be strong selection pressure against mutant cells. Possibly this furthered the maintenance of haploidy, as well as the correlation of diploidy with more complex development (Otto and Orive 1995; this point is also developed in ►Chap. 6). In tetraploids like the agronomic crop alfalfa, mutations are hidden, but selfing results in inbreeding depression. This may be due to loss of third-order interactions or homozygous deleterious recessive alleles (Jones and Bingham 1995).

The molecular clock Notwithstanding the substantial variation in mutation patterns among species, it was recognized several decades ago that the average rates of amino acid or nucleotide substitution are approximately constant *for a particular protein or gene* among taxa (Kimura 1987). This fairly steady accumulation in sequence divergence over time has been called the ‘molecular clock’ (Zuckerandl and Pauling 1965; Wilson et al. 1977; Kumar 2005). Presumably, it reflects the fact that the same functional constraints exist for a given gene or gene product in different organisms. Clock analysis involves comparing the amino acid or gene sequences in a particular highly conserved protein (such as hemoglobin), or base sequences in a given gene, in several species. For protein comparisons, each discrepancy is assumed to represent a stable change in a codon corresponding to the altered amino acid. In this analysis, the original (ancestral) state, being extinct, is of course unavailable for comparison. So the versions of the sequence that appear in two or more living relatives of the phyla of interest are compared and expressed as units of accumulated amino acid or nucleotide changes. The organisms are then arranged in a branching diagram drawn to minimize the number of changes needed to describe all the permutations from the ancestral sequence. The number of stable genetic changes can then be related to chronological estimates of evolutionary time, obtained from fossil records, since the species diverged from the common ancestor

(recall ►Chap. 1). At a detailed level, molecular clocks have proven in general to give more resolution and reliability than the fossil record (cf. ►Chap. 1).

How and why the rates have stabilized where they have is unknown. An inference is often made that the clock is centrally standardized, ticking away uniformly for all species, much like a radioactive decay process, but this is not strictly the case (Jukes 1987). Substitutions are nonlinear such that irregularities occur and these may or may not reconcile over time. Also, for species of both microorganisms and macroorganisms, the clock can tick at different rates among genes, even when only silent substitution rates are considered (Sharp et al. 1989). Nevertheless, among other interesting comparative observations are the following by Ochman and Wilson (1987): (i) the silent mutation rate for protein-coding genes in *Salmonella typhimurium* and *Escherichia coli* is comparable to that in the nuclear genes of invertebrates, mammals, and flowering plants; (ii) the average substitution rate for 16S rRNA of bacteria is similar to that of 18S rRNA in vertebrates and flowering plants; (iii) the rate for 5S rRNA is about the same for bacteria and eukaryotes.

To summarize, the present indications are that the rates of DNA divergence for a gene encoding a given function are approximately the same regardless of the organism. From this generality we derive not only very useful phylogenetic information but also insight into the origin of sequence variations and how gene families diverge (Kumar 2005).

Finally, it should be noted that the rate of phylogenetic change evidently is not controlled by the mutation rate. Rather, the evolutionary history of the major groups of organisms appears to depend on ecological opportunities afforded the simple or complex genetic variants (Wright 1978, pp. 491–511). Evolution, overall, has been for increased size and complexity, although there are several exceptions (►Chap. 4). Evolutionary rates, unlike mutation rates, vary greatly even along single phyletic lines. For some existing genera (e.g., the lungfishes and the opossum among animals; horsetails [*Equisetum*] and club mosses [*Lycopodium*] among plants), evolution has been virtually at a standstill for hundreds of millions of years (Wright op. cit.). Where genetic variation has provided for a major and entirely new way of life, swift adaptive radiation follows. Examples include the development of feathers, wings, and temperature regulation in the case of the birds; efficient limbs, hair, temperature regulation, and mammary glands in the mammals. Among prokaryotes, genes can be transmitted horizontally and in blocks among distant relatives with major evolutionary implications; see later discussion with respect to bacteria in ►Sect. 2.3. Other ecological opportunities are presented when new forms colonize an area where either the niches are unoccupied or are better filled by the mutant than the wild-type (Wright 1978).

2.2.2 Recombination

Recombination is a process leading to the rearrangement of nucleotides by the breaking and rejoining of DNA molecules. There are three general categories (Alberts et al. 2015): homologous; conservative site-specific; and transpositional. The term ‘homologous’ in this context pertains to regions of sequence similarity (homology) between the DNA molecules involved. As noted earlier, modifications induced by processes such as transposition, or in aberrant homologous recombination, are also considered to be forms of mutation.

General or homologous recombination involves genetic exchange between members of a pair of homologous DNA sequences (i.e., those of extensive sequence similarity). Its most widespread role is in the accurate repair of double-strand breaks but it also is involved in genetic exchange between two paired chromosomes in eukaryotes (or DNA strands in the

case of prokaryotes; Lengeler et al. 1999). Recombination or crossing-over does not occur with every chromosome at every meiosis.

Where recombination is part of the formal meiotic process in eukaryotes, it is frequently referred to as **meiotic recombination** and includes (i) independent or Mendelian assortment of entire maternal and paternal homologs during metaphase I and anaphase I of meiosis; and (ii) reciprocal recombination of chromosome segments (crossing-over) that occurs between the nonsister chromatids of paired homologs during prophase I of meiosis (Alberts et al. 2015). In this role, recombination is both a mechanical process to insure the equivalent segregation of chromosomes to the two daughter germ cells, as well as a mixing process that reassorts genes on those individual chromosomes. Although genes may be reassorted, gene order on the chromosomes involved usually remains the same, since the recombining sequences are quite similar (cf. transposition, below). As alluded to earlier, an analogous phenomenon also occurs in bacterial transformation and conjugation, where exogenous chromosome fragments are integrated into the genome of the recipient cell by homologous recombination (see Sex, and Adaptive Evolution in Prokaryotes in ► Sect. 2.3). Thus, features of homologous recombination are common to all organisms. For eukaryotes, assortment is quantitatively more significant than crossing-over in all organisms with a haploid chromosome number exceeding two (Crow 1988). Chromosomes in all organisms can break spontaneously. An additional key role (nonmeiotic) of homologous recombination is to accurately repair such single- or double-strand breaks. (Double-strand breaks can also be corrected by a cruder mechanism known as nonhomologous end-joining, which reseals the DNA but results in a mutation where the strands broke; Alberts et al. 2015.)

Transposition and conservative site-specific recombination differ from general recombination in that diverse, specialized segments of DNA are moved about the genome (Alberts et al. 2015). The pieces moved, which vary considerably from a few hundreds to many thousands of nucleotide base pairs, go by various colloquial ('jumping genes'; 'selfish DNA'), as well as specific names. For example, in bacteria, there are two general types of transposable elements: (i) **insertion-sequence or IS elements** that can move themselves but do not carry genes other than those related to the movement; and (ii) **transposons**—carry the movement genes as well as others (Griffiths et al. 2015). Transposition and conservative site-specific recombination differ in the reaction mechanisms involved and because the conservative process requires that there be specialized DNA sequences on both donor and recipient DNA, whereas transposition generally requires these specialized sequences only on the transposon.

Transposition is further divided into three classes involving (i) DNA-only transposons; (ii) retroviral-like retrotransposons; and (iii) nonretroviral retrotransposons. Transposons are typically 'cut' from one place and 'pasted' into another place in the genome without duplication, whereas the retrotransposons (retroposons) are duplicated because they are transcribed into RNA, reverse-transcribed into DNA, and then reintegrated. Different kinds of TEs predominate in different kinds of organisms: bacterial transposons tend to be the DNA type, whereas **it has been estimated that about half of mammalian genomes originate from TEs primarily of the retroelement group** (Van de Lagemaat et al. 2003). Similarly, 64% of the genome of the powdery mildew pathogen *Blumeria* consists of TEs (Spanu et al. 2010). At least four identical transposon families occur in invertebrates and vertebrates, where they have moved horizontally from the former to the latter evidently by parasite-host interactions (Gilbert et al. 2010).

TEs have broad evolutionary implications because they can mutate existing genes, create new genes, and affect gene regulation at both the transcriptional and post-transcriptional

levels when they insert nearby (see Epigenetics below; also Slotkin and Martienssen 2007; Freschotte 2008; Elbarbary et al. 2016). The resulting variation can be adaptive and, for example, IS have been shown to promote the evolution of specialists (as opposed to generalists; see ►Chap. 3) in controlled growth experiments with bacteria (Zhong et al. 2004, 2009). Frequently, they have deleterious consequences because the insertion and rearrangement can lead to disease (e.g., the Alu elements in humans; Kazazian 2004). For this reason and because the elements replicate themselves independently of host chromosomes, they have been called ‘intra-genomic parasites’ or ‘selfish DNA’ (Charlesworth 1985; also see ►Sect. 2.3), though such terms are simplistic and potentially misleading (Kidwell and Lisch 2001). Moreover, repetitive DNA may well have a functional role in the physical ordering of the genome (Shapiro and von Sternberg 2005). McClintock (1956) discovered TEs in the late 1940s and 1950s in maize, and called them ‘controlling elements’ because, although distinct from genes, they could modify gene expression. They have since been well documented in many other taxa including bacteria (phage Mu; insertion sequences; transposons conferring antibiotic/metal resistance or surface antigen variation); yeasts (Ty and mating type elements of *Saccharomyces*); and animals (*Drosophila* transposable elements and hybrid dysgenesis determinants; vertebrate and invertebrate retroviruses). At least in maize, and presumably in other macroorganisms, transposition occurs at predictable times and frequencies in the ontogeny of the individual. In maize, a controlling element can have a similar effect on genes governing different biochemical pathways and at different places in the genome (McClintock 1956; Fedoroff 1983, 1989). Moreover, a single element can control more than one gene concurrently.

Both transposition and site-specific recombination are complex processes in detail and a molecular biology text such as Alberts et al. (2015) should be consulted for specifics. **The key conceptual point is that these phenomena occur broadly if not universally and add considerable genetic versatility or plasticity to organisms beyond the conventional mechanism of recombination normally associated with sexuality.** For additional comments, see Genomic Plasticity and Epigenetics sections, below.

An analogous process pertains to integration of some **plasmids** (small, ancillary, self-replicating extrachromosomal elements) into the bacterial chromosome and ‘promiscuous’ (organellar) DNA into the nuclear chromosomes. To date, plasmids (see Sect. 2.3) are known to occur ubiquitously in bacteria. Though uncommon in eukaryotes, they are found in many fungi and in some higher eukaryotes, often in association with mitochondria (Funnell and Phillips 2004). Promiscuous DNA has been detected in most eukaryote species examined, including plants, filamentous fungi, yeasts, and invertebrates (Timmis et al. 2004). The term originated with Ellis (1982) for DNA that appeared to move from chloroplasts to mitochondria. Subsequently, evidence has accrued for a broader process, including the insertion of mitochondrial and chloroplast DNA sequences into nuclear DNA (Herrmann et al. 2003; Matsuo et al. 2005; Bock and Timmis 2008). The extent to which such transpositions produce functional transcripts remains unclear; for example, most of the plastid DNA engulfed by the nucleus may be eliminated by genome shuffling (Matsuo et al. 2005). If the genes are expressed, there are potentially significant evolutionary implications because of the different modes of inheritance of a nuclear as opposed to an organellar gene. Presumably such transpositions also can interrupt nuclear gene function, depending on where they insert.

To what extent is mobile DNA favored by natural selection? At the level of the ‘selfish gene’ (Dawkins 1989) selection is presumably for these mobile elements, especially in eukaryotes with excess DNA, or in bacteria where they add unique (useful but generally described

as nonessential) features as plasmids, discussed later under prokaryote recombination. However, once essential gene functions are disrupted, selection at the level of the gene will be offset by counter-selection at the level of the physiological individual. So the tendency should be toward some balance in opposing forces. Note, however, that deleterious genes can still spread in a population by over-replication or if they alter reproductive mechanisms to favor themselves (Campbell 1981; Chap. 6 in Bell 1982). Certain TEs (retroviruses, below) provide an independent mechanism for moving genetic material horizontally.

Perhaps the most intriguing subcategory of site-specific recombination involves the retroviruses. They are unique in having an RNA genome that replicates by reverse transcription through a DNA intermediate, which can then integrate as provirus into host chromosomal DNA. Retroviruses are considered with transposons because of similar structure and functional properties. They do not transpose in the same way that bacterial transposons do, but are analogous in that they can be viewed as intermediates in the transposition of viral genes from proviral integration sites in the host chromosomes (Varmus 1983; Varmus and Brown 1989). Retroviruses and viral-like elements have been described from diverse genomes, including those of mammals, the slime mold *Dictyostelium*, yeast, fish, reptiles, birds, and plants (McDonald et al. 1988). The most information is on mammalian retroviruses and because of the interesting evolutionary implications, this is summarized briefly below (see also Doolittle et al. 1989).

There are two retroviral categories (Benveniste 1985; Varmus 1988): *Infectious* or *exogenous* retroviruses occur as a few copies of proviral DNA per cell, only in the genome of infected cells; they are infectious and often pathogenic (as in HIV); and they are transmitted horizontally, i.e., among individuals rather than from mother to daughter. *Endogenous* retroviruses occur as multigene families in the host DNA of somatic cells (and occasionally germline cells, in which case they are transmitted vertically) of all animals of the species of origin (Benveniste 1985; Jern and Coffin 2008). They have been viewed as fossil representatives of retroviruses extant in the geological era when they entered the germline (Jern and Coffin 2008). About 7–8% of the human genome is of retroviral origin (Jern and Coffin 2008). Endogenous retroviruses are usually not infectious to cells of the species of origin, but are often so to those of other species. In fact, it is this property of being able to replicate in heterologous cells that sets them apart from conventional cellular genes.

Both types of retroviruses can cause host genes to mutate, or can carry host genes with them. This is significant because of the spreading of somatic variation through the soma, and the possibility of introducing variation directly to the germline (►Sect. 2.5). **From an evolutionary standpoint, the endogenous group is particularly intriguing because some members have been transmitted horizontally and, once established, may subsequently have been incorporated into the germ line and transmitted vertically** (i.e., from mother cell-to-daughter cell and from parent to offspring). Benveniste (1985, p. 362) reviews evidence that retrovirus transfers have included those “*from ancestors of primates to ancestors of carnivores, from rodents to carnivores, from rodents to primates, from rodents to artiodactyls, from primates to primates, and from primates to birds.*” A specific example is the baboon type C viruses, which are transmitted vertically in primates and which were transferred millions of years ago to ancestors of the domestic cat where they were incorporated into germ cells and inherited thereafter in conventional Mendelian fashion (Benveniste and Todaro 1974, 1975). Benveniste (1985) proposes that retroviruses may promote genetic interaction above the species level, much as do plasmids in bacteria (discussed later).

Are retroviruses a major force in evolution? They do seem to play a major role by influencing gene regulation (McDonald 1990) and by their phylogenetic implications noted

above. The analogue that comes closest to this is the transfer and incorporation of bacterial DNA into plant chromosomes. Crown gall and hairy root diseases of plants involve transfer of a plasmid (tumor-inducing or Ti plasmid) from a pathogen, *Agrobacterium tumefaciens*, to the plant, where a fragment (T-DNA) is covalently integrated into the host nuclear genome. In essence, the agrobacteria use genetic engineering methods to force the infected plant to synthesize nutrients (opines), which the bacteria utilize (discussed in ►Chap. 3; Zambryski 1989; Platt et al. 2014). (Although the process of *Agrobacterium* oncogenesis is often considered with processes involving transposable elements, strictly speaking the T-DNA is not a transposon because it does not jump about the chromosome. The fascinating *Agrobacterium*/plant tumor story is summarized in ►Chap. 3.)

2.2.3 Genomic Plasticity

The sorts of nucleotide changes reviewed above emphasize that both protein-coding and non-coding regions are subject to dynamic evolutionary change. Cells can read multiple messages from the same DNA sequence and these do not just pertain to protein structure (Shapiro 1999, 2002). Mutations are not limited to nucleotide substitution but can be genome-wide rearrangements involving potentially large blocks of nucleotides. As Shapiro states (2002, p. 9) “... *living cells can rearrange their genomes in any way that is compatible with the rules of DNA biochemistry.*” Such rearrangements allow rapid genotypic and phenotypic changes by organisms, as in the response of microorganisms to antibiotic selection pressure discussed later, or by the vertebrate immune system to novel antigens. Physical changes in the genome accomplished by the rearrangements are amplified by DNA interactions with cellular complexes that do not alter the sequences (►Table 2.2 and Shapiro 2002; see Epigenetics below). The bacterial genome in particular is highly plastic, with multiple mobile components of the genome interacting, being transferred, gained and lost in a dynamic equilibrium (Touchon and Rocha 2016; developed in Sect. 2.3).

2.2.4 Epigenetics and Gene Regulation

Epigenetic controls include nongenetic, enzyme-mediated chemical modifications of DNA structure (methylation of DNA residues after replication) and changes to the associated protein (mostly histones) (Feng et al. 2010; Griffiths et al. 2015). Both processes affect transcription and thereby gene activity. Some alterations to histone as well as DNA methylation marks can be inherited stably and such instances are referred to as ‘epigenetic inheritance’ (although terminology varies; see Eichten et al. 2014). Epigenetic changes such as erasure of DNA methylation or ‘reprogramming’ can also occur in both plants and animals where they play an important role in development (Feng et al. 2010). Classic cases of epigenetic inheritance include gender-specific gene silencing even though both the maternal and paternal copies are functional (‘genomic imprinting’), and even the silencing of an entire chromosome (random inactivation of one of the two copies of X chromosomes in female mammals).

It is perhaps in the realm of gene regulation more so than any other that Shapiro’s (2002) computational metaphor of the genome, noted above, is most apt. The inert DNA storage medium (hard drive) interacts with cell complexes to format the information in a readable, transmissible manner. Though complex in detail, regulation of the message takes many forms

Table 2.4 Six points at which eukaryotic gene expression can be regulated (Alberts et al. 2015). Bacteria generally employ similar mechanisms (see Chaps. 3 and 7)

Type of control	Overview of mechanism
1. Transcriptional	When and how often a gene is transcribed ^a
2. RNA processing	How the RNA transcript is spliced/processed
3. RNA transport/localization	Which completed mRNAs in cell nucleus are transported to cytosol and where localized
4. Translational	Which mRNAs are translated by ribosomes
5. mRNA degradation control	Selective destabilization of certain mRNAs
6. Protein activity control	Selective activating, deactivating, degrading or compartmentalizing certain proteins

^aThere are multiple controls at this level. One form, chemical changes to DNA (e.g., methylation) or histones altering gene function but not DNA sequence, is termed ‘epigenetic’

and is exerted at numerous points broadly directed at the transcription and post-transcription levels (Table 2.4). In bacteria, one method of control involves proteins that inhibit or activate transcription of specific genes (see discussion of the *lac* operon in Chap. 3). In eukaryotes, transcription is controlled in part by multiple *cis*-regulatory sequences, so-named because they typically are located on the same DNA molecule as the gene affected. In plants, chromatin modifications and other forms of genetic regulation influence development and reaction to environmental stimuli (Feng et al. 2010; Eichten et al. 2014). In animals, there may be 5–10 times as many such regulatory modules as there are genes (Davidson 2006). Sequence-specific DNA-binding proteins (transcription regulators), which are themselves variably active by time in the life cycle and place in the organism, read this information and determine the time and location of genes to be transcribed (Davidson 2006; Tuch et al. 2008; Gilbert and Epel 2009).

It appears from the model organisms studied to date that most if not all the eukaryote genome is transcribed and among the products are several classes of small, noncoding RNAs (ncRNAs) (Amaral et al. 2008). Two such classes of ncRNAs are termed microRNAs (miRNAs) and small interfering RNAs (siRNAs) (Amaral et al. 2008; Ghildiyal and Zamore 2009). They appear to be primarily regulatory, achieving their effect by interacting with transcription factors, RNA polymerase, or directly with DNA (Amaral et al. 2008). For example, transcription can be affected in various ways, including through various modifications of chromatin structure (Slotkin and Martienssen 2007; Figueiredo et al. 2009). The miRNAs direct mRNA degradation or repress translation (Amaral et al. 2008) and may function at multiple hierarchical levels in regulatory networks (Makeyev and Maniatis 2008; Dekker 2008). Among the siRNAs, the so-called *exo*- and *endo*-forms (Ghildiyal and Zamore 2009) are derived from dsRNA and are associated with Argonaute (Dicer) proteins that execute the regulation. This specific form of silencing, differing in details but broadly represented among eukaryotes, is known as **RNAi (interference)** (Ghildiyal and Zamore 2009). At least in plants, it serves also as a form of antiviral defense (Baulcombe 2004). As a regulating mechanism on gene expression, RNA interference (RNAi) is a topic of intense research on gene silencing

and has practical implications, for example, in disease and pest control (Zhang et al. 2015). Morphological evolution may depend largely on changes in gene expression accomplished by mutations in regulatory networks (Prud'homme et al. 2006, 2007; Davidson 2006; Carroll 2008), though the extent to which such changes drive evolution is controversial.

The far-reaching impact that seemingly minor or innocuous changes to the genome can have is evident in the following example: There is a class of genes (proto-oncogenes) that appears to have a normal housekeeping function within the cell but which, if altered by mutation in a coding or noncoding region, can lead to malignant transformation. In the human *Ha-ras* gene, a single point mutation within the fourth intron can cause a tenfold increase in gene expression and transforming activity (Cohen and Levinson 1988). This is only one example of many that show *there are several ways to change gene expression without changing the message itself* (see also McDonald 1990). Undoubtedly, numerous ways of altering expression are important in evolution and may explain why humans have so many genes in common with other organisms. It was pointed out insightfully by King and Wilson in 1975 that probably humans differ from chimpanzees largely because of differences in gene expression, rather than in gene structure. As the human genome continues to be studied intensively in the years since being sequenced in 2001, it has been a surprise to find that the protein-coding regions, some 3 billion bases in all, account for only a trivial amount (about 1.5%) of the total. At least some and perhaps much (still actively debated) of the vast noncoding portion—often formerly derided as ‘junk DNA’—now appears to have a critical regulatory function.

Finally, that there are many gene copies in the more complex life forms provides the opportunity for alterations in the genome by changing introns, exons, or both. New gene products can be exposed to natural selection while the organism is buffered through continued function of the unaltered product encoded at another site(s). Genes that have been rendered silent can be retrieved, and other genes turned on or off, all with the phenotypic expression of a point mutation in a coding region, but accomplished simply by changing patterns of regulation. Controlling the timing or extent of gene expression has important ecological implications because this mechanism in effect increases the phenotypic plasticity of the organism as discussed in some detail in ►Chap. 7. Cells of more complex organisms in particular have a large repertoire of mechanisms to generate genetic variability!

2.3 Sex and Meiotic Recombination

2.3.1 Definitions, Origin, and Maintenance

Definitions of sex vary considerably (Michod and Levin 1988). Here it will be considered to be the bringing together in a single cell of genes from two (or rarely more) genetically different genotypes or individuals (Maynard Smith 1978b). In most but not all organisms, sex is tied to reproduction, i.e., to the production of a new individual that arises from the zygote or its functional equivalent. The most important consequence of sex is the acquisition by the zygote of new genes (mutations in the germ cells of one or both parents) and new gene combinations. The latter arise from reciprocal exchange (crossing-over) of genes between homologous chromosomes, and reciprocal exchange of chromosomes (independent assortment) during meiotic recombination as part of gametogenesis.

Processes that are sexual are general (though maybe not universal; see ►Sect. 2.4) among the prokaryotes and eukaryotes. Among extant prokaryotes, genetic exchange can occur in

three ways (discussed in detail later in the next section): (i) by direct uptake of DNA from the environment under specific conditions (transformation); and as mediated by (ii) phage infection (transduction) or (iii) plasmids (conjugation). Transformation is likely very ancient. Arguably, it can be traced as far back as the emergence of protocells in primordial communities of progenotes that existed ca. 4.0–3.6 billion years ago, prior to the divergence of the Last Common Ancestor of the three lineages Archaea, Bacteria, and Eukaryotes (see ►Chap. 4). Woese (2002), among others, has postulated an era dominated by the lateral transmission of information with communities as a whole varying in descent and selection operating largely at the level of community optimization.

Meiotic sex, traceable to the last eukaryotic common ancestor (Speijer et al. 2015), is postulated to have arisen from mitosis (Wilkins and Holliday 2009). It may have begun by the interposition of one new step (homolog synapsis) followed by ‘parameiosis’ in the occasional diploid protocells within an otherwise haploid cell population of early protists. It is perhaps vestiges of this innovation that remain today in ‘parasexual’ cell cycles, most notably among the fungi, as discussed later. Over evolutionary time, sex cells, and sexual fusion arose with enhanced inter-genic recombination during the pairing step, as well as related meiotic properties such as synaptonemal complexes. With meiosis thus began the classic alternation of generations, haploid/diploid phases of the eukaryotic life cycle (►Chap. 6). Wilkins and Holliday (2009), however, reason that mitosis originated before meiosis because it occurs universally among the eukaryotes, whereas meiosis is both more complex and, though very widely represented as noted, is not universal.

But what was the major selection pressure for the evolution of sex and the origin of meiosis? While increased recombination would imply the creation of new, potentially favorable gene combinations and disruption of unfavorable ones (see below), this feature alone is not generally regarded as conveying sufficiently immediate benefit to constitute a potent evolutionary force. It conveys instead future benefits to the population or lineage rather than to the individual. An alternative, interesting hypothesis (one of many) is that meiosis facilitates repair of DNA damage (Bernstein et al. 1985). If damage occurs to only one DNA strand, it can be repaired by using the other strand as template. However, when both strands are damaged, correction requires proximity of the homologous chromosome for a template and a process akin to recombination. A variation of the Bernstein argument is that sex arose in prokaryotes as a side-effect of processes to promote DNA replication and repair. Indeed, much of the biochemical machinery and the underlying genes are homologous, including the RecA family of so-called recombination enzymes and their eukaryotic homologs (Cox 1999; Marcon and Moens 2005). Notwithstanding the name (‘Rec’ for recombination), DNA replication and repair appear to be their primary function (Redfield 2001). In the broader context of the implications of sex for endogenous and exogenous repair, Stearns (1992, p. 183) says that “... the evolution of sex can be viewed as the evolution of the mechanisms preventing the ageing of the germ line.”

For eukaryotes, Wilkins and Holliday (2009) have modified the Bernstein repair hypothesis by arguing that the benefit of meiosis was not in restoration of the original wild-type DNA message but prevention of recombination-induced injury. Consequently, meiosis improved recombinational accuracy and confined the process to a localized period in the cell cycle (while also likely increasing the frequency of genetic recombination mainly among the ‘right’ sequences; Wilkins and Holliday 2009). Recombination is error-prone because the ‘wrong’ or ectopic pairing may occur leading to various irregularities in the message including deletions, duplications, or aneuploidy. The invention of homolog synapsis in meiosis would have

enforced accurate alignment so that only identical regions were in register, not diverged homologous sequences elsewhere on the chromosome.

The foregoing may explain why sex arose but, having arisen, why is it maintained in some form in virtually all taxa? The fact that sexual reproduction is ubiquitous yet carries significant costs is commonly referred to as the paradox of sex and has been called by Bell (1982, p. 19) “*the queen of problems in evolutionary biology*.” This controversial matter has been debated in countless papers, review articles, and books (e.g., Williams 1975; Maynard Smith 1978b; Bell 1982; Michod and Levin 1988; Otto and Lenormand 2002; Rice 2002; Otto 2009). At the risk of trivializing a very complex issue, the following general points can be made in passing. In eukaryotes the costs, relative to an alternative of asexual reproduction, are various but principally of three sorts: (i) in anisogamous species (i.e., those in which the male and female sex cells contribute unequally in terms of gamete characteristics to the production of progeny) the twofold ‘cost of producing males’, since only females produce offspring; (ii) in sexual eukaryotes, the twofold ‘cost of meiosis’, or more accurately the cost of genome dilution, since each parent’s genes are diluted by one-half in their progeny; and (iii) the disruption of favorable gene combinations resulting from past selection, analogous to deciding to reshuffle your hand of cards when you already have a good hand in a game of poker (Otto 2009). With respect to (i), a significant general distinction between eukaryotic microorganisms—many species of which are single celled—as opposed to macroorganisms, is that the former typically are isogamous. In such cases there is no ‘cost of producing males’.

Against these handicaps of sexual reproduction is set the traditionally acclaimed advantage, namely the ability to combine beneficial alleles from different individuals, restoring variation that would otherwise become dissipated in asexual reproduction (Otto 2009). Simultaneously, in changing environments, sex also would eliminate genetic associations that may have been favorable in a previous selective environment but are no longer so (Otto 2009). An example of such oscillating conditions as they influence coevolving species is that sex can produce novel genotypes that enable lineages of macroorganisms to survive attack by much shorter lived (hence more rapidly evolving) microbial parasites. Conversely, asexually reproducing lines would be vulnerable both because the parasite quickly evolves virulence to overcome host resistance genes, and because as the size of the host clone increases from generation to generation it presents a progressively larger target. This is one form of the ‘Red Queen Hypothesis’ (Hamilton 1980; Clay and Kover 1996; Lively and Morran 2014).

Through sex, deleterious mutations that would otherwise accumulate in a finite population in the absence of recombination (Muller’s ‘ratchet’ 1964; Bell 1988a) also are purged. These advantages accrue over time (Rice 2002). Crow (1994) has shown conceptually how sexual species can in effect clump harmful mutations and eliminate several at once by a mechanism such as truncation selection (i.e., selection eliminating all individuals beyond a certain phenotypic state or value). In contrast, asexual species can only eliminate them in the original genotype in which they occur. Also, in outbreeding sexual species, genes influencing the mutation rate will become separated from the corresponding mutations, whereas they will not in asexual species (Drake et al. 1998), so evolutionary processes may be quite different in the two situations. A recent test of the longstanding dogma that sex accelerates adaptation, executed by comparing evolutionary events in sexual and asexual populations of *Saccharomyces cerevisiae*, confirms that sex acts by providing a sorting mechanism to separate the beneficial from the deleterious mutations: advantageous mutations are combined into the same background, whereas deleterious mutations are separated from advantageous backgrounds that would otherwise carry them to fixation (McDonald et al. 2016).

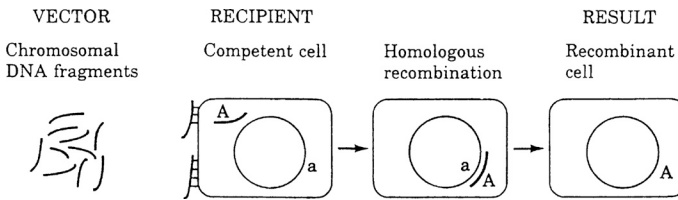
The above arguments do not imply that sex necessarily increases variation or that such variation necessarily increases fitness (Otto and Lenormand 2002; Otto 2009). So while competitively superior genotypes can be produced, sexual recombination overall may not have a net advantage. While an earlier generation of models suggested fairly constrained conditions wherein sexuality would be maintained, recent evolutionary models (Otto 2009) run under more realistic conditions imply the evolution of sexuality when (i) selection varies over time (past genetic associations no longer favorable); (ii) selection varies over space (where migration-driven genetic associations are locally disadvantageous); (iii) rates of sex as opposed to asexual reproduction are relatively high for less fit individuals and relatively low for fit individuals, i.e., when facultatively sexual individuals in poorer condition allocate more resources to sexual reproduction; and (iv) populations are finite. With respect to (iv), most evolution-of-sex models are deterministic and assume infinitely large populations. This is not realistic and can lead to the wrong conclusions because the best genotype can be lost by drift in finite populations. Sexual recombination would allow it to be regenerated relatively quickly whereas asexuality would not. For discussion of this and the other conditions, see Otto (2009).

2.3.2 Sex and Adaptive Evolution in Prokaryotes

Not only do bacteria engage in sex, but if one means by the term that genes from different sources are recombined in a single entity, then “*bacteria are particularly sexy organisms*,” to use the words of Levin (1988), and subsequently, with colleagues (Johnsen et al. 2009) that “... *Bacteria may not have sex often, but when they do it can be really good, at least evolutionarily speaking*”¹. A distinctive attribute of bacterial sexuality is that sex is not formally linked to reproduction, whereas in eukaryotes there is a linkage. Prokaryotes reproduce asexually (clonally and in most species, though with notable exceptions [Angert 2005] by binary fission, see later section and Fraser et al. 2007) and the current consensus is that they undergo recombination sporadically. An extension of Levin’s wry comments would be that, the degree of bacterial sex apparently varies considerably among populations and species, ranging from the arguably relatively promiscuous *Neisseria* to the relatively asexual *Pseudomonas syringae* (Sarkar and Guttman 2004) or *Salmonella* (Maynard Smith et al. 1991, 1993; Feil and Spratt 2001; however, see Tibayrenc and Ayala 2015). Overall, recombination now appears to be the norm rather than the exception, at least among pathogens (Maynard Smith et al. 2000; Touchon et al. 2009; Bobay et al. 2015).

Unlike recombination in macroorganisms that characteristically involves two complete genomes, recombination in prokaryotes is asymmetrical, typically involving a relatively large and a small donation, respectively, from the two partners. This entails replacement of small nucleotide regions in the recipient cell by corresponding regions moving almost always in unidirectional fashion from the donor bacterium. Furthermore, prokaryotic sex involves, in addition to chromosomal genes, various accessory genetic elements differing in their degree of mobility and autonomy, ranging from phages, plasmids, and transposons at the high end to genomic islands and integrons¹ at the other (Levin and Bergstrom 2000; Touchon and Rocha

1 Genomic islands are discrete DNA inserts presumptively acquired horizontally that encode various functions such as those involved in symbiosis or pathogenicity (Hacker and Kaper 2000). Integrons are gene expression elements that capture promotorless genes by site-specific recombination from external sources, thereby converting them to functional genes. All consist of three parts: (i) an attachment site; (ii) a gene encoding an integrase; (iii) and a promoter directing transcription of the captured genes (Mazel 2006).



■ **Fig. 2.1** Gene transfer in bacteria by acquisition of free DNA (transformation). Incoming chromosomal fragments from the environment (*dark lines*) bind to bacterial cell (*rectangle*); one enters the bacterium and is incorporated into the genome (*light circle*) by homologous recombination. From Levin (1988); reproduced by permission of Sinauer Associates, Inc., Sunderland, MA ©1988

2016). These and related mobile elements have been called a “*motley riff-raff of DNA and RNA fragments*” (Dawkins 1982, p. 159). The ability of bacteria to routinely accept DNA from other species and even entire genes and gene clusters appears to far exceed the capability of eukaryotes in doing so.

While the three processes involved in prokaryotic sex—transformation, transduction, and conjugation—are distinct from each other and from eukaryotic sex, all produce effectively the same end result: acquisition of and usually recombination of DNA from genetically different individuals (cells). Each mechanism is quite complicated in detail and beyond the scope of this discussion. The synopsis here is intended to provide a basis for comparisons between prokaryotic and eukaryotic sex; specifics are available in general microbiology texts and advanced treatises (e.g., Levin 1988; Neidhardt et al. 1990; Bushman 2002; Madigan et al. 2015). Regardless of the process, the entering DNA may either (i) become degraded by restriction enzymes; (ii) replicate by itself (if it has its own origin of replication, as in the case of phage or plasmids) or; (iii) recombine with the recipient’s chromosome by homologous recombination. Occasionally, it may recombine as mediated by phage integrases or mobile element transposases, or by various ‘illegitimate’ or nonhomologous means such as by double-strand break repair (Ochman et al. 2000). These latter mechanisms pertain particularly to incorporation of sequences by horizontal gene transfer. In practice, because of the limitations of detection methods, it may not be known which of the three processes is responsible for recombination in a given situation. The presence of synteny (gene blocks similarly arranged in the species compared) within and surrounding the genome break points, as well as absence of viral- (phage) related sequences, is usually sufficient to eliminate transduction. If the bacterium is naturally transformable (below), it is very difficult to separate transformation from conjugation.

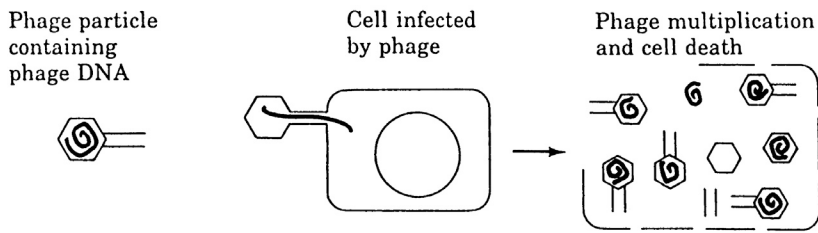
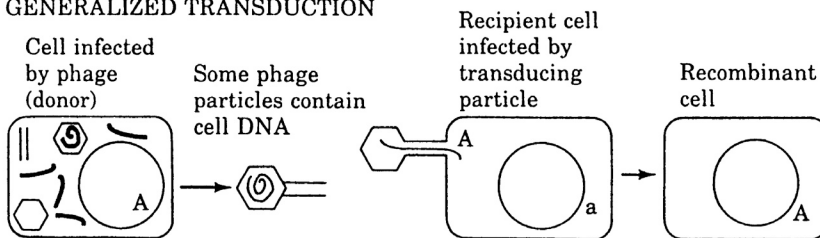
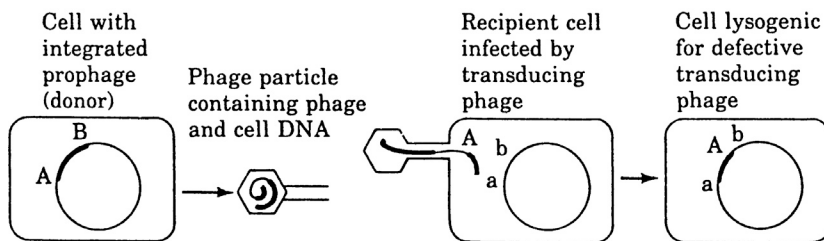
In **transformation**, a bacterial or archaeal cell takes up naked DNA from the surrounding medium (originating usually from a lysed or decomposing cell) which is then integrated into and replicates with the recipient’s genome (■Fig. 2.1). The amounts of genome transferred vary over an order of <1–100 kilobases. The mechanism hinges on several conditions, among them development of a transient ability (competent state) by the recipient to be transformed. Competency is a complex trait and the selection pressures for the genes involved are unclear (Levin and Cornejo 2009; Johnston et al. 2014) though recognition and uptake of foreign DNA are highly evolved processes. Transformation occurs naturally and has been documented in many genera, including *Streptococcus*, *Staphylococcus*, *Hemophilus*, *Neisseria*, and *Pseudomonas* (Levin 1988; Madigan et al. 2015). Even within such genera only certain species and strains are transformable and under specific conditions. For instance, in

B. subtilis, competency occurs in a small percentage of cells as they enter stationary phase of growth and is a stochastic phenomenon. It is one of several examples of bistability (see ►Chap. 7 and Dubnau and Losick 2006).

In nature, transformation would appear to be potentially most significant in habitats where DNA in dead and lysed cells can be protected from digestion (e.g., by adsorption to a matrix such as clay particles) and where cells occur densely, as in biofilms (see, e.g., Hall-Stoodley et al. 2004; Hiller et al. 2010). Some authors have speculated that transformation may not even be primarily a sexual process but rather has evolved to provide the cell with nutrients (Redfield 2001), though this opinion has been challenged (Johnston et al. 2014). Others postulate that its most important function is to acquire genes from without as a source of variation (Levin and Bergstrom 2000) and specifically to restore ('reload') genes lost or degraded in a local population though still present in the overall population at large or metapopulation (Szollosi et al. 2006). Under simulation conditions, Redfield (1988) showed that even when the acquired DNA is from dead cells, transformation can reduce mutational load and transformed populations had a higher mean fitness than asexual populations. It is also noteworthy that competent, nongrowing cells may have a transient selective advantage over noncompetent, dividing cells under episodic conditions that kill growing cells (Johnsen et al. 2009). The striking evolutionary dexterity of the human pathogen *Streptococcus pneumoniae* has been attributed to gene transfer among strains by transformation (Hiller et al. 2010; Croucher et al. 2011). This is not only interesting from the standpoint of basic microbial ecology but has important practical implications for understanding the pathogenesis and epidemiology of diseases caused by *S. pneumoniae*. The genomic analysis shows that this lineage evidently acquired both drug resistance and evolved adaptations (antigen switches) to counter vaccine pressure multiple times (Croucher et al. 2011). This bacterium is a natural resident commensal of the human nasopharynx and also exists as a potentially invasive pathogen. It habitually causes ear infections of children as well as frequently fatal infections such as meningitis, bacteremia, and pneumonia.

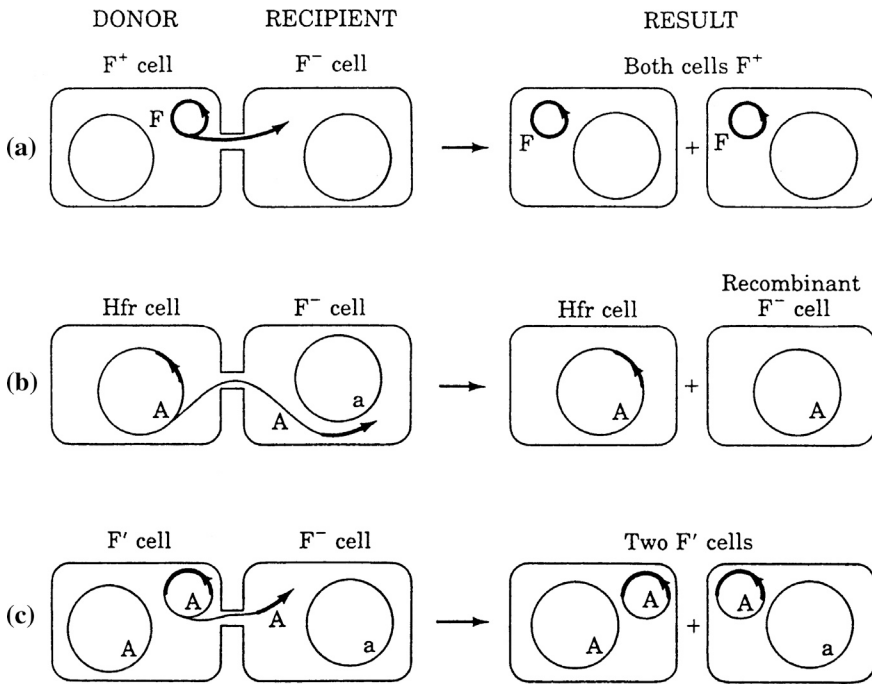
Transduction occurs when bacterial DNA is packaged within the protein capsid of a bacteriophage particle and injected into a recipient cell during the viral infection process (Levin 1988; Madigan et al. 2015; Salmond and Fineran 2015). In generalized transduction, chromosomal genes from the donor bacterium are transferred when a small proportion of progeny phage carry some random portions of bacterial DNA instead of phage DNA. The donor's genes must recombine with homologous sequences in the recipient's chromosome, otherwise they will be lost. In specialized transduction, temperate phage move specific, adjacent bacterial genes when the occasional phage genome excises imprecisely from its latent or prophage state in the bacterial chromosome at the onset of the lytic cycle (■Fig. 2.2). Transduction has been shown to occur in numerous genera of soil and aquatic bacteria under nonsterile experimental conditions, though not all bacteria are transducible and not all phages can transduce. Nevertheless, since many phage can infect diverse bacterial species, DNA can be moved across significant evolutionary distances. Transduction is a fortuitous process, essentially resulting from mistakes in phage growth.

Conjugation involves cell-to-cell contact and is controlled by genes carried on certain so-called 'conjugative plasmids'. The result is transmission from donor to recipient of plasmid (extrachromosomal) DNA alone or, occasionally, both plasmid and various lengths of chromosomal DNA (■Fig. 2.3). Furthermore, there is a diverse group of mobile genetic elements maintained largely as part of the chromosome that can also be excised and transferred to another cell during conjugation but which, unlike plasmids, cannot replicate autonomously.

(a) NORMAL PHAGE INFECTION**(b) GENERALIZED TRANSDUCTION****(c) SPECIALIZED TRANSDUCTION**

■ **Fig. 2.2** Three possible results of infection of bacteria by bacteriophage (transduction). *Heavy lines* = phage genome; *light lines* = host genome. **a** In normal phage infection by lytic phages, replication of the phage leads to packaging of phage DNA in phage particles. **b** In generalized transduction, a few of the progeny phage contain random portions of bacterial DNA instead of phage DNA. These progeny phage then transfer the host DNA into new cells where it replaces the recipient's genes. **c** In specialized transduction, part of the phage genome is replaced by adjacent host genes when the phage excises from the bacterial chromosome; these genes are inserted by site-specific mechanisms when the virus enters the genome of its new host bacterium. From Levin (1988); reproduced by permission of Sinauer Associates, Inc., Sunderland, MA ©1988

These are collectively referred to as 'integrative and conjugative elements' (Wozniak and Waldor 2010). The best known of the conjugative plasmids is the F (for fertility) plasmid of *E. coli* and closely related enteric bacteria. One of the proteins specified by a cluster of F genes is for the sex pilus, a temporary projection that joins the F^+ cells and F^- cells and through which plasmid DNA moves. The result of mating is two F^+ cells. In rare F^+ cells (known as Hfr = high frequency of recombination), where the F particle is integrated into the bacterial chromosome, chromosomal genes also are transferred. The F particle can also mobilize a class of nonconjugal plasmids when both occur in the same donor cell. However, to put these events in perspective, Hfr formation, even under laboratory conditions, is a comparatively rare event. Such Hfr 's, once formed, are relatively unstable because the F factor excises



■ **Fig. 2.3** Three mechanisms for gene transfer in bacteria as mediated by F plasmids (conjugation). *Heavy lines* = plasmid genome; *light circle* = host chromosome. Donor and recipient cells are shown united by the pilus bridge and the results of the conjugation events are indicated. **a** F plasmid conjugation: Only a copy of the F plasmid is transferred but not incorporated into the bacterial chromosome. **b** Hfr-mediated transfer: The F plasmid is part of the donor host chromosome (in Hfr cells, see text). During conjugation, a copy of some of the donor chromosome is transferred and may replace part of the recipient's chromosome. **c** F' (prime) transfer: In rare cases the F plasmid may excise from the chromosome carrying some of the host chromosome along at which point it becomes known as an F' plasmid. When conjugation subsequently occurs, it can transmit the host genes it acquired as well as itself. In this case, the plasmid may not integrate into the host chromosome and the recipient becomes diploid for the newly acquired chromosomal genes. From Levin (1988); reproduced by permission of Sinauer Associates, Inc., Sunderland, MA ©1988

at a high frequency. Therefore, the contribution of this type of genetic exchange to variation of bacterial populations in nature is unclear. While the Hfr's may be a relatively rare phenomenon, transfer of the F plasmid is not, the process occurring rapidly and efficiently and rapidly spreading the plasmid infectiously within a population (see **Sidebar**). In another rare phenomenon, recombination may occur between a site on the F⁺ plasmid and a site on the host chromosome resulting in what becomes known as a F' (F prime) plasmid containing host genes. When an F' mates with an F⁻ cell usually the entire F plasmid is transferred and the result is a partial diploid, i.e., for the genes carried on the F plasmid as well as on the recipient's chromosome. Plasmids can also be transferred by transduction and transformation as well as by conjugation.

As alluded to earlier, **plasmids** are ubiquitous among bacteria (Funnell and Phillips 2004; Touchon and Rocha 2016) and constitute the most common form of semi-autonomous replicating pieces of DNA (so-called replicons). Bacterial cells may carry more than 20 of these elements. Some may be very large (on the order of Mb DNA) and differ little from second-

ary chromosomes (see footnote under summary below and Touchon and Rocha 2016). In the older literature those plasmids that could integrate into the chromosome were called episomes. Bacteria lacking them generally multiply normally under laboratory conditions; hence, plasmid DNA seemingly does not encode essential functions and may be best regarded as a desirable albeit expendable source of accessory traits. However, ‘essential’ should be qualified as what appears to be nonessential under laboratory conditions, where such assessments are made, may well be essential in nature. Moreover, exchange of key genes among replicons and the chromosome may well lead to acquisition of essential genes by such elements and thereby persistence in the bacterial lineage.

To the extent to which plasmids contribute to bacterial competitiveness, they also benefit themselves indirectly by enhancing their representation in the population of bacterial carriers. Plasmid DNA contributes to the genetic plasticity of the carrier and confers many characteristics of adaptive value in diverse environments. The best known of these is antibiotic or heavy metal resistance (R factor plasmids; see **Sidebar**); others include the ability to induce tumors in plants (*Agrobacterium tumefaciens*), nitrogen-fixing capability (*Rhizobium* spp.), increased virulence (*Yersinia enterocolitica*), and antibiotic synthesis (*Streptomyces* spp.). Transfer of plasmids has been observed between bacterial strains, species, and, in some instances, even between unrelated genera (Funnell and Phillips 2004). Remarkably, in the case of plant tumors (the crown gall disease, above), the mobilization function of a bacterial plasmid promotes its transfer to *plant* hosts: Not only can this plasmid move among bacteria, but plants have access to the gene pool of at least some bacteria (Buchanan-Wollaston et al. 1987; McCullen and Binns 2006). While plasmids may be the key means by which bacterial genes are transferred in nature, it is not yet clear that conjugation is the mechanism involved, although this is generally inferred to be the case.

SIDEBAR: A Case Study: Transferable Drug Resistance in Bacteria

A central message of this chapter is that although all living things have generally analogous means of generating and transmitting genetic variation, the potential evolutionary rates of microorganisms are much higher than those of macroorganisms. This is a function of their short generation times, hence large population sizes (► Chap. 4), widespread dissemination and mixing, efficient means of genetic exchange, numerous accessory genetic elements, and the relatively broader phylogenetic range over which gene exchange occurs.

A classic example of natural selection in action (evolution in changing environments) is the phenomenon of antibiotic resistance in bacteria. Such resistance is either encoded by chromosomal genes or plasmid-borne (genes typically on one class of conjugative plasmids known as resistance or R factors) (Nikaido 2009). Plasmids are especially significant ecologically because resistance to several different antibiotics can be combined in a single element, which can also serve a role as an efficient vector as well as mediating genetic rearrangement (Koch 1981; Sandegren and Andersson 2009). In the presence of an antibiotic, the plasmids may increase in size due to gene duplication and/or in plasmid copy number per cell; i.e., the antibiotic resistance genes can be amplified when necessary and deamplified when not needed (Sandegren and Andersson 2009). Early stages in the evolution of resistance of *E. coli* to ciprofloxacin involve extensive cytological changes, including the production of multi-chromosome-containing filaments (Bos et al. 2015).

In the presence of an antibiotic(s), the drug-resistant phenotype obviously has a selective advantage. In absence of the drug, there are typically fitness costs associated with

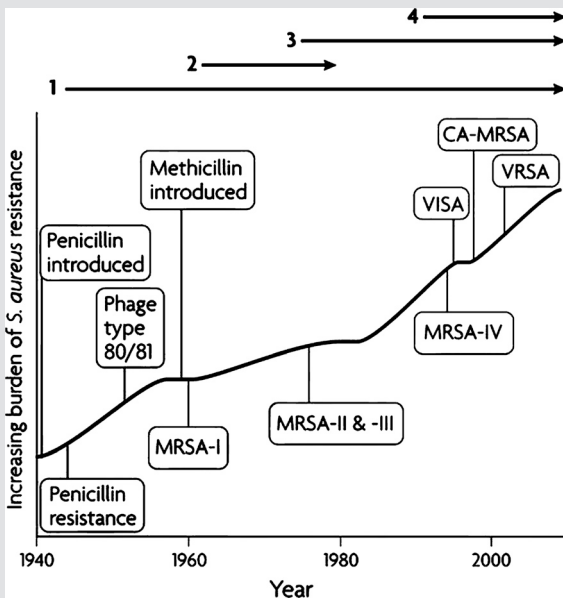
resistance manifested as a decline in growth rate or virulence, but bacteria often respond by compensatory mutations at other loci or amplification of the affected gene (Gagneux et al. 2006; Andersson and Hughes 2010). Amelioration of fitness costs in the absence of the drug commonly result in a bacterial population that is fitter in drug-free culture than the uncompensated resistant population, but less fit than the original wild-type. Levin et al. (2000) speculate that the common ascent of intermediate-fitness compensated mutants, rather than high-fitness revertants, may be attributable to higher rates of compensatory mutation relative to reversion and to bottlenecks in culture associated with serial passage. From a microbial ecology standpoint, as well as with respect to the strategy of antibiotic administration, the fact that reversion to sensitivity is difficult has important implications (e.g., Tanaka and Valckenborgh 2011).

Resistance to many if not most antibiotics is known, and frequently the genes responsible are carried within transposons. Conjugative plasmids typically replicate during transfer; hence, acquisition by the recipient is not at the expense of loss from the donor. The resistance genes spread so quickly (often exponentially) that the phenomenon has been called *infectious* drug resistance. For instance, following the introduction of antibiotic therapy with streptomycin, chloramphenicol, and tetracycline from 1950 to 1965 in Japan, the proportion of drug-resistant *Shigella* (the bacterium that causes bacillary dysentery) increased from about 1–80% of the isolates (Mitsuhashi 1971). The history of the use of various antibiotics to control *Staphylococcus aureus* is analogous. Waves of resistance dated from the introduction of penicillin in the 1940s, through other β -lactam antibiotics, in particular methicillin. *S. aureus* can cause rapidly progressing, potentially fatal skin and soft-tissue infections. This situation has culminated with worldwide epidemics of methicillin-resistant *Staphylococcus aureus* (MRSA) clones that spread rapidly among healthy individuals, as opposed to earlier outbreaks associated mainly with hospitals and similar settings (Chambers and DeLeo 2009) (■ Fig. 2.4).

In a parallel situation, the genomic plasticity and hence evolutionary versatility of *Streptococcus pneumoniae* to multiple antibiotic and vaccine pressure has been thoroughly documented. This was approached by large-scale genomic analysis of a single ancestral strain (clone) by sequencing 240 isolates of the lineage PMEN1 from 22 countries (Croucher et al. 2011). Their approach allowed relatively easy determination of the number and size of base replacements, and thereby in separating variation due to recombinations from point mutations. The clone has diversified rapidly since its estimated origin in 1970. Base substitutions occurred about once every 15 weeks; though recombination events occurred at about one-tenth that rate, they introduced an average of 72 single nucleotide-polymorphisms each, with 5% of the replacements involving more than 30 kb of genome. Overall, 74% of the genome length had received a recombination event in at least one isolate. On average, some 74,000 bp of sequence were affected by recombination in each strain. A related intensive study of recombination events among four strains in the nasopharynx in a single patient repeatedly sampled over a much shorter period (7 months) confirms the rapid evolutionary potential of *S. pneumoniae* (Hiller et al. 2010). At least 156 kb corresponding to about 7.8% of the genome was exchanged, probably by transformation, during the multiple recombination events. The authors suggest that this case supports the ‘distributed genome hypothesis,’ which proposes that: (i) bacterial species consist of multiple, co-occurring, complementary strains among which the fluid ‘pangenome’ or species-level genome is distributed (Ehrlich et al. 2010); and (ii) pathogen

genetic diversity accomplished by concurrent infection with multiple strains (so-called polyclonal infection) can overwhelm a host's immune response (Hiller et al. 2010).

The activity of diverse mobile genetic elements in the origin and spread of resistance genes provides an interesting case study in the plasticity of bacterial evolutionary processes.



■ **Fig. 2.4** Four major epidemic waves of antibiotic resistance in *Staphylococcus aureus* with the respective eras shown above the graph. Wave 1 began in the mid-1940s shortly after the introduction of penicillin, driven by strains producing a plasmid-encoded penicillinase. The second wave following within 2 years after the introduction of methicillin in 1959 was caused by a strain designated here as MRSA-I (for methicillin-resistant *S. aureus*) that carried a gene encoding a low-affinity penicillin-binding protein that confers resistance to the entire class of β -lactam antibiotics. Wave 3 began in the mid-late 1970s with clones derived from earlier infections as well as new lineages; the use of vancomycin to treat MRSA infections led to vancomycin-intermediate *S. aureus* (VISA). Wave 4 began in the mid-late 1990s and marked the emergence of MRSA strains with distinct attributes in the community at large (CA-MRSA) as opposed to clinical settings. Strains tolerating even higher doses of vancomycin are called vancomycin-resistant *S. aureus* (VRSA). From Chambers and DeLeo (2009); reproduced from *Nature Reviews Microbiology* by permission of Macmillan Publishers Ltd. ©2009

To the role of conventional point mutation in molding the genome must now be added such dynamic processes and factors as phage transduction, transposons, plasmids, horizontal gene transfer, pathogenicity islands, and probably others awaiting discovery. Transfer of drug resistance is not only an important phenomenon within the context of basic microbial ecology, but obviously has profound implications in practical terms of how antibiotics can be over-prescribed in medicine. Furthermore, because drug-resistant bacteria of animal origin can cause serious diseases in humans (Wegener 2003), the routine use of antibiotics as animal feed supplements should be restricted. For additional reading on the ecology of transferable drug resistance, see Pr nting and Andersson 2011; Jackson et al. 2011).

The evidence has been reviewed (Levin 1988; Touchon and Rocha 2016) for plasmid- and phage-mediated bacterial sex being simply coincidental to the infectious (parasitic) transfer of the elements involved and the availability of recombination repair systems in the bacterial hosts. The population biology of the highly mobile elements such as plasmids prompts many interesting questions as to the selection pressures favoring their survival and genetic options for the host bacteria. For example, since plasmids impose at least a minimal fitness cost on the host, it should be advantageous from the plasmid's perspective to maintain genes that are at least occasionally beneficial to the bacterium. Likewise, there are some situations where it may be more or less advantageous for the bacterium to have its genes either integrated into the chromosome or on a mobile (infectiously transmitted) vehicle (Bergstrom et al. 2000).

In an historical context it worth a note in passing that key aspects of bacterial sexuality were discovered by François Jacob at the Pasteur Institute in the 1950s and 1960s, who, with his colleague, Elie Wollman, devised a simple but elegant technique for mapping genes in a linear sequence. Using a Waring blender to interrupt mating bacterial cells at sequential times during the process, they found that different genes were transferred in a specific order and could be mapped as to position. This also revealed the circular nature of the bacterial chromosome and that episomes can be added to or subtracted from the bacterial chromosome (see especially Chap. IX in Jacob and Wollman 1961). For this and related brilliant work on gene regulation and his ingenious deductions, Jacob shared the 1965 Nobel Prize in Physiology or Medicine with his colleagues Jacques Monod and André Lwoff. (Jacob's Nobel lecture is well worth reading, as is his eloquent autobiography, *The Statue Within*.)

Bacterial sexual reproduction, as reviewed above, serves to propagate the variation in existing genes by so-called vertical transmission (in the course of cell division) and incorporation through homologous recombination of such novelties as mutational changes, gene rearrangements, and related intra-genomic alterations (Gogarten et al. 2002). An important extension of the sexual process called **lateral or horizontal gene transfer (HGT)** (Ochman et al. 2000), alluded to in various earlier contexts, involves the integration within a recipient cell of entire genes or gene clusters that are fundamentally new (exogenous) to the genome. **Unlike vertical transmission, in HGT the donor and recipient may be distantly related prokaryotic species or genera, at the extreme even in different domains** (for transfers from prokaryotes to eukaryotes or among eukaryotes see Dunning Hotopp et al. 2007; Keeling and Palmer 2008; Andersson 2005, 2009; Gilbert et al. 2010). The sequences transferred tend to retain characteristics of the donor and so can be distinguished from ancestral DNA (for a discussion of how such inferences are made, see Ochman et al. 2000). Traits introduced by HGT frequently are complex phenotypic features including antibiotic resistance, virulence, and metabolic properties.

HGT has profound implications for organism taxonomy and phylogeny as well as evolutionary ecology. With respect to the former, prokaryotic species boundaries (Rossello-Mora and Amann 2001; Rosen et al. 2015) tend to become blurred (Ochman et al. 2005; Shapiro et al. 2012). Thus, bacterial species might better be thought of less rigidly as “*distinctive arrays of varying but co-adapted gene complexes which are periodically reshuffled*” (Duncan et al. 1989, p. 1586). Perhaps more significantly, the evolutionary history of a gene does not necessarily equate with that of the organism. This is most dramatically seen in the contentious debates surrounding attempts to root the universal tree of life (Brown 2003; Chaps. 4 and 27 in Barton et al. 2007; ► Chaps. 1 and 4 of this text). A quantitative measure of the prevalence of lateral transfer is provided by Lawrence and Ochman (1997), who examined a sequenced region of about 30% of the *E. coli* chromosome. Based on atypical base composition and

	Utilize lactose	Utilize citrate	Produce H ₂ S	Produce indole	Produce urease	Lysine decarboxylase	Lifestyle
<i>Escherichia coli</i>	+	-	-	+	-	+	Mammalian commensal
<i>Shigella flexneri</i>	-	-	-	+	-	-	Primate pathogen
<i>Salmonella enterica</i>	-	+	+	-	-	+	Mammalian pathogen
<i>Klebsiella pneumoniae</i>	+	+	-	-	+	+	Soil
<i>Serratia marcescens</i>	-	+	-	-	+	+	Soil
<i>Yersinia pestis</i>	-	-	-	-	-	-	Mammalian pathogen

■ **Fig. 2.5** Representative phenotypic properties of, and evolutionary relationships among, certain enteric bacteria based on nucleotide sequence data (+ = presence; – = absence of the trait). Many such species-specific traits are conferred to bacteria, not by conventional mutation or the reshuffling of existing genetic information, but by the acquisition of sequences through horizontal gene transfer (HGT) from distant relatives. Note in particular the differences between *E. coli* (mammalian commensal) and its sister species *S. enterica* (mammalian pathogen). These traits and others are inferred to have arisen from HGT. From Ochman et al. (2000); reproduced from *Nature* by permission of Macmillan Publishers, Ltd. ©2000

codon usage patterns, they estimated that at least 17% of the protein-coding sequences resulted from HGT since the divergence of *Escherichia* from *Salmonella* (or more than 600 kb of transferred DNA accumulated at the rate of about 31 kb per million years; smaller estimates have been made in other systems). This was viewed as being quantitatively similar to the amount of variation introduced through mutation. However, qualitatively, as alluded to above, the impact of the two processes is very different, with HGT potentially providing novel functions to the recipient, i.e., a significantly changed phenotype, immediately, and ultimately being a process that enhances genome dynamics and diversifies lineages (Nowell et al. 2014). Indeed, as an extreme example, it has been proposed recently that adaptation of *Archaea*, which were originally hyperthermophiles, to a mesophilic lifestyle is attributable to HGT from the *Bacteria* (López-García et al. 2015).

The relationships among several enteric bacteria are shown in ■ Fig. 2.5. Frequently, the key traits defining those relationships have resulted from genes transferred by HGT. For example, *E. coli* acquired the lactose operon, hence the ability to utilize lactose and thereby colonize the intestinal tract as a commensal, while genes conferring pathogenicity islands in *Salmonella* and *Shigella* also are conveyed horizontally (Ochman et al. 2000; Gogarten et al. 2002). So lineages can separate as a result of HGT and entirely new niches are created as opposed to being simply refined. The implications can be visualized in terms of Wright's classical model (1932) portraying a population at successive peaks on an adaptive landscape. Having climbed a peak, an evolving population would tend to stay there because to descend would mean to decline in fitness. As Gogarten et al. (2002) point out, peaks may never be explored if they can be reached only by changing one gene at a time (i.e., by mutational processes). HGT can overcome this constraint by introducing multiple changes simultaneously.

To summarize, sexual reproduction and adaptive evolution in prokaryotes is loosely analogous to that in the sexual eukaryotes with some important qualifications. Unlike most eukaryotes, the prokaryotes reproduce for the most part asexually (clonally) as discussed further under 2.4 The Asexual Lifestyle, later. Clones tend to progressively diverge genetically by the sequential accumulation of mutations. Of course they also diverge as a result of recombination or acquisition of foreign DNA from distant phylogenetic sources by horizontal transfer. Whether at the point of acquiring foreign DNA from close or distant sources a cell is still considered to be a clonal member is a matter of definition; see later section and Tibayrenc and Ayala (2012). Bacteria are considered to be haploid because generally there is only one copy usually of one chromosome present.² Any genetic change is thus immediately expressed and exposed to natural selection. Genes are exchanged over a substantially wider phylogenetic range and routinely in the prokaryotes unlike the case among eukaryotes. This provides both for the variation in existing genes typical of eukaryotes (by homologous recombination) but, more significantly, the wholesale introduction of unique traits by HGT and nonhomologous recombination allowing major changes in organism habitat or niche. Moreover, the sexual process is mediated by a diverse array of semi-autonomous accessory genetic elements that do not appear to play a significant role in eukaryotes.

Selection pressure on microorganisms, and prokaryotes in particular, typically favors a small genome and rapidity of reproduction. The evolution of prokaryotic chromosome structure and evolution of genome organization are separate but closely related and interdependent processes (Touchon and Rocha 2016). The genome is a streamlined, plastic, dynamic one where there is tension between high plasticity at one extreme and high organization at the other. One gets the impression of a busy railroad station, a scene of frenetic activity with the continuous comings and goings trains and throngs of passengers who intermingle but in an organized manner. Though the population size of prokaryotes typically is vast, dwarfing that of eukaryotes, the effective genetic size can be lower because of bottlenecks or the recurrent selective sweeps of mutants through a population (see comments on asexuality, later, and Reeves 1992; Maynard Smith et al. 2000; Levin and Bergstrom 2000).

2.3.3 Sex and Adaptive Evolution in Some Simple Eukaryotes: The Fungi

For the fungi, sex followed by meiosis is generally similar to that in the higher eukaryotes. Three distinctions with broad ramifications, however, need to be emphasized: (i) Variation among taxa in rapidity of completion of the stages of sexual reproduction—plasmogamy, karyogamy, and meiosis—establishes four basic life cycles and their associated nuclear conditions, in addition to a category putatively ascribed as nominally asexual. These patterns are summarized below along with some relevant terminology. (ii) Fungi show extreme phenotypic plasticity and can exhibit alternative or supplementary genetic systems, most notably

2 While this is correct as a generality, bacteria may be polyploid and some species even have >100 copies of the chromosome per cell. Additionally, some bacterial species have more than one type of chromosome, typically of different sizes. In such cases the smaller chromosomes are called secondary chromosomes or chromids. Some bacterial lineages (e.g., *Burkholderia* and closely related genera) are characterized by variable numbers of chromosomes, while others (*Vibrio* spp.) consistently (i.e., over geological time) carry two distinct chromosomes. For details and implications, see Touchon and Rocha (2016).

heterokaryosis and parasexuality (also reviewed briefly below). Thus, for the fungi, recombination need not be meiotic or sexual (for details see Taylor et al. 1999a,b, 2015; Billiard et al. 2012). A given fungal species or individual may display different modes of sexuality based on seasons of the year or geographic locations (Taylor et al. 1999a). Furthermore, both sexual and vegetative fusions enable information molecules such as plasmids and mitochondrial DNA to be exchanged among fungal thalli. The septa (cross-walls) transversing the hyphae are rarely complete in areas other than where reproductive structures are borne. Apart from the obvious physiological implications pertaining to cytoplasmic streaming, solute movement, and so forth, this means that multiple, potentially genetically distinct nuclei exist within a common cytoplasm. (iii) Sex is associated often with resting spore formation and is frequently triggered by adverse environmental conditions (see ►Chap. 7). For these reasons and because the fungi display the genetic characteristics of a transitional group between the prokaryotes and the more complex eukaryotes, the following comments provide background informative for discussions in later chapters.

Life cycles of the fungi and associated nuclear states, mating systems, and degree of genetic variation are numerous and typically complex (Billiard et al. 2012). Simplistically, the options might be categorized as follows (Carlile et al. 2001): (i) **Asexual**—an artificial assemblage of species (formerly, Fungi Imperfecti or Deuteromycota) united historically by their apparent absence (based on morphology) of conventional sexuality. Existence of a sexual cycle has recently been documented or very strongly implied from genomic or population genetics data for the human pathogens *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus fumigatus*, all traditionally believed to be strictly clonal (Heitman 2006, 2010; Taylor et al. 2015). Mycologists abandoned this formal classification in 2012; the current concept is that fungi exhibit both episodes of recombination and clonality in their life cycles. More is said later about this category in the later section The Asexual Lifestyle. (ii) **Haploid (haploid/monokaryotic)**—the life cycle is predominately haploid and the hyphal cells generally uninucleate (monokaryotic). Karyogamy follows soon after plasmogamy. Meiosis and compartmentation of the meiotic products by septa in the hyphae follow soon after that (Ascomycota), or, if delayed, the zygote remains dormant (Zygomycota, e.g., the bread mold *Rhizopus stolonifer*) (iii) **Haploid (haploid/dikaryotic)**—the cycle is similar to (ii) except that each cell of a dikaryotic mycelium “... contains paired, synchronously dividing nuclei, one of each given by the original gametic genotypes” (Anderson and Kohn 2007, p. 345; see below). Classic examples are the rust fungi such as *Puccinia graminis*, discussed in ►Chap. 6. The dikaryotic phase may be transient (typical of the Ascomycota) or exist for much of the vegetative phase where karyogamy is delayed after plasmogamy (common in the Basidiomycota). For example, typically in the mushroom-producing (agaric) fungi, the perennial dikaryotic mycelium grows indefinitely and hidden from view as a saprobe in the soil or thatch layer. It may give rise annually to a short-lived (days or weeks) flush of mushrooms in which the life cycle stages of karyogamy followed immediately by haploidy (meiosis and sporulation) occur. The dikaryotic, vegetative, mycelial phase of the well-known fairy ring mushrooms common in pastures may exist for several centuries, with the rings expanding progressively outwards (Dix and Webster 1995). (iv) **Haploid/diploid**—the cycle alternates regularly or irregularly between these two nuclear conditions as in many yeasts. (v) **Diploid**—this group overlaps with (iv) and is analogous to most higher organisms where the haploid phase is relatively inconspicuous and may be relegated to the gametes. It includes members of the Oomycota (fungus-like organisms now considered to be a monophyletic group within the kingdom Straminipila; Webster and Weber 2007). The vegetative cells and much of the life cycle of

many yeasts are diploid (Chaps. 10 and 24 in Webster and Weber 2007) or preponderantly so. For example, the remarkable morphogenetic gymnastics of certain strains of the human pathogen *Candida albicans*, formerly thought to be a bland and well behaved, 'obligate diploid'; classic yeast, illustrate how dynamic are the reproduction options of the fungi (Hickman et al. 2013). It is the variations on the theme that are informative.

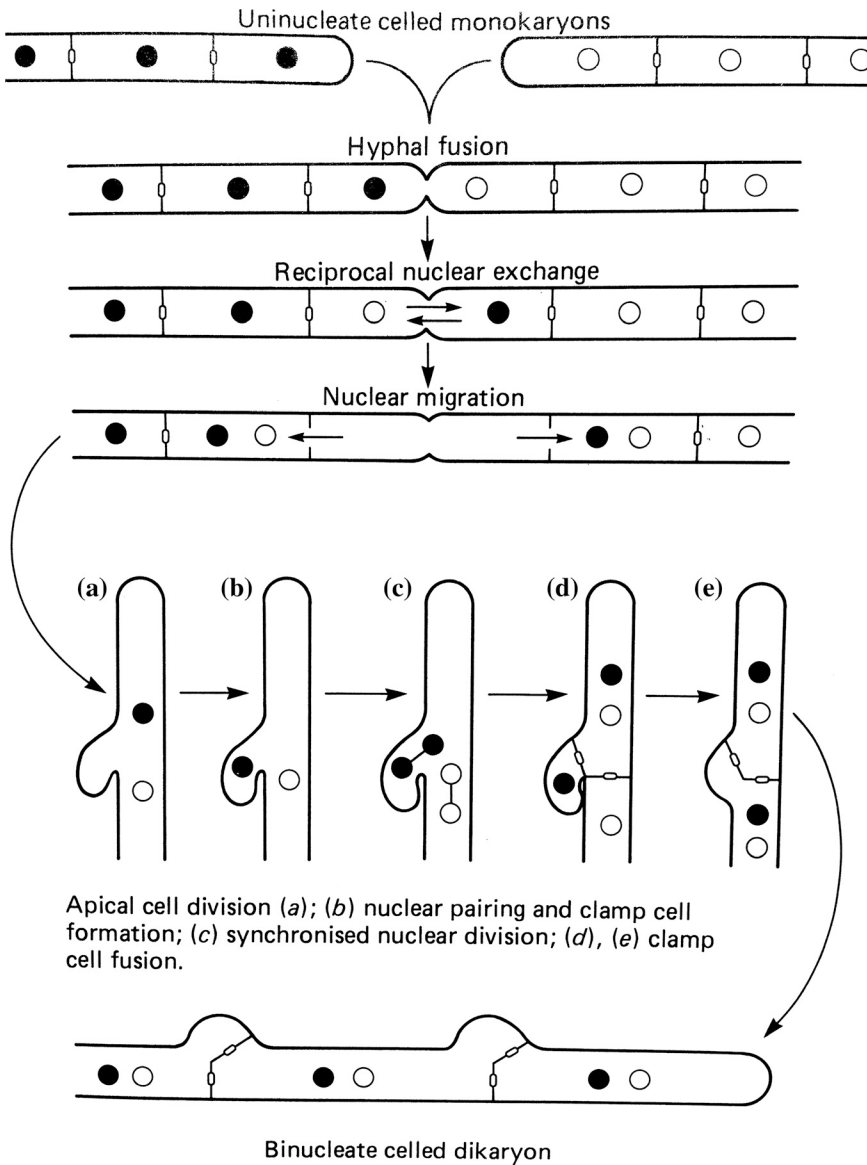
Most fungi belong either to category (ii) or (iii), that is, they reproduce both sexually and asexually and are haploid for most of their life cycle. The haploid, asexual phase of the cycle is generally repeated numerous times annually, typically by rounds of sporulation but in some taxa by other asexual methods such as by budding or fragmentation of the soma. The sexual phase normally occurs only once a year, and may more or less overlap the asexual state. Although a cycle comprised of haploid and to greater or lesser extent diploid structures is thus conventional in the fungi, the alternation of generations is not distinct or regimented, unlike the case in many higher organisms.

Some fungi that engage in sex are hermaphroditic in that a single thallus can function simultaneously as both 'male' and 'female'; such organisms are thus self-fertile, nonoutcrossing, and are said to be **homothallic**. Others are self-sterile, requiring the union of two compatible thalli and are said to be **heterothallic**. Across the fungal world and including the related Oomycetes, the range of sexuality includes haploid selfing, diploid selfing, and outcrossing (for details see Billiard et al. 2012). Fungi have at least two mating types and in the mushrooms there are as many as several thousand (Brown and Casselton 2001).

Dikaryosis, heterokaryosis As noted in the life cycle overview above, the dominant vegetative phase characteristic of the phylum Basidiomycota is generally a dikaryotic ($n + n$) mycelium. This prolonged, balanced nuclear phase is unique in the living world to certain fungi. As such it warrants some discussion.

In the basidiomycetes, typically the post-meiotic, haploid sexual spore (basidiospore) germinates to produce a filament (hypha) containing genetically identical (therefore, homokaryotic) nuclei in uninucleate or monokaryotic cellular compartments. Hyphae tend to fuse constitutively as they grow; such fusions serve several physiological purposes as well as setting the stage for nuclear transfer in sexually or vegetatively compatible colonies (Glass et al. 2004). When such anastomoses involve different but genetically very closely related individuals of sexually compatible mating type, a developmental program is triggered that results in the dikaryotic mycelium on which the fruiting bodies later develop (Anderson and Kohn 2007; Webster and Weber 2007). This process is dictated, at least in the mushrooms, by different allelic versions of multiallelic genes at two unlinked loci, *A* and *B*; Casselton and Economou 1985; Brown and Casselton 2001). The *B* genes encode pheromones and pheromone receptors; the *A* genes encode proteins involved in transcriptional regulation and synchronized division and cellular distribution of the conjugate nuclei described below. In the 'dikaryotization' process, each homokaryon acts simultaneously as male and female, both donating and receiving nuclei, which divide and migrate quickly and generally more or less widely throughout the recipient mycelium under control of the *B* genes. However, the mitochondria typically do not migrate, so the resultant dikaryon has a consistent nuclear background but is a spatial mosaic for cytoplasmic content, including mitochondrial DNA.

The classic dikaryon appears in the form of sexually compatible nuclei representing the original gametic genotypes, physically associated and dividing synchronously for an indeterminate period as growth ensues (■ Fig. 2.6). Cell divisions in the extending hyphal apex typically are associated with construction of a cytological feature known as the clamp connection, which ensures that the daughter compartments receive exactly two nuclei, one of each mat-



■ **Fig. 2.6** The sequence of events leading to a fungal dikaryon from two compatible monokaryons. From Casselton and Economou (1985); reproduced by permission of Cambridge University Press, ©1985

ing type. Throughout this protracted dikaryotic phase, the conjugate nuclei remain separate but physically associated and in close molecular communication—intriguingly, the distance between them has an impact on gene expression (Anderson and Kohn 2007). The two nuclei may exchange genetic material and undergo somatic recombination (Clark and Anderson 2004; Gladfelter and Berman 2009). When dikaryotization is complete, the new genetic entity functions as a unique genetic individual and tends to rebuff through somatic incompatibility further fusions and genetic invasion by other dikaryons.

What are we to make of this fascinating quirk of nature? The expansive dikaryotic phase in the basidiomycetes may represent little more than a remnant in a general evolutionary trend to diploidy. Alternatively, this life cycle phase may be under ongoing positive selection pressure as a means for these fungi to have the phenotypic plasticity to cope with heterogeneous environments. There is some theoretical support as well as limited experimental evidence (Clark and Anderson 2004) to suggest that the latter is the case. Raper and Flexer (1970, p. 419) refer to dikaryosis as “... *something of a biological oddity and an evolutionary cul-de-sac, although a highly successful one* ...” and elsewhere (p. 417) that “*the stable vegetative dikaryon is not only the physiological and genetic equivalent of a diplophase, it is a far more plastic and adaptable consortium of two genomes than is the diploid.*” Casselton and Economou (1985) speculate that the phenomenon of extensive bidirectional migration of nuclei allows them to be positioned for sexual fusion even where cytoplasmic incompatibility occurs (see discussion in ►Chap. 5). In population genetics terms, J.L. Harper (University of Wales; personal communication, 1987) has likened the phenomenon of dikaryotization to mate competition theory. Among polygamous animals there is often intense competition between males for females, which has led to the evolution of various sexual selection strategies. Analogously, in the fungi it would seem advantageous for an organism to preempt rivals by sequestering the nuclei of a compatible mate.

There are, however, even further variations on this theme of genetic versatility. They include ‘sectoring’, which may occur among dikaryons restoring the monokaryotic state locally. The occasional exchange of nuclei among dikaryons has been reported, as well as the more common phenomenon of a dikaryon mating with and thereby dikaryotizing a monokaryon if it is of compatible mating type. The most noteworthy complication is that many basidiomycetes (as well as ascomycetes) form not strictly dikaryons but rather heterokaryons with multinucleate cells (James et al. 2008). In this fluid situation the numbers of nuclei per cell are variable. The nuclei are not associated in pairs and their activities may or may not be coordinated. Thus, nuclear ratios of the parental genotypes are imbalanced (not 1:1 as in dikaryons) and nuclear competition and altered allele frequency occur in sections of a mycelium where the frequency of one nuclear type outnumbers the other. Indeed, in a fungal syncytium (multiple nuclei within a common cytoplasm) there may be thousands or even millions of nuclei of various origins, each of which is more-or-less mobile for potentially long distances, i.e., throughout the syncytium. Movement of nuclei largely by bulk cytoplasmic flow may reach several $\mu\text{m/s}$. Each different nucleus has the potential to give rise to a new individual (Roper et al. 2011, 2013). Fusion phenomena with respect to inter-individual compatibility or repulsion, the multinucleate condition, and fungal cytology are discussed further in ►Chap. 5.

Heterokaryosis clearly provides for additional adaptive flexibility (for example to changing environments) beyond that afforded by the more regulated conditions of dikaryosis or diploidy, and it has other implications. Different cells or nuclei within the mycelium may differ in ploidy or by having undergone mitotic recombination (see parasexual cycle, below). Genomic ‘conflict’ occurs where selection acts in opposition at different levels. For instance, at the organelle level, selection could favor a particular nuclear type, yet disfavor the resultant heterokaryon nuclear ratio at the level of the mycelium as a whole (James et al. 2008). Different nuclear ratios have been reported for conidia versus mycelium of the basidiomycete *Heterobasidion parviporum*, indicating that nuclei may compete to be included in these asexual propagules (James et al. 2008; Roper et al. 2011). Conflict also

arises because of the different degrees and modes of somatic transmission of the nuclear and mitochondrial genomes—the nuclei being relatively freely exchanged between the homokaryons whereas the mitochondria in the resultant dikaryon being contributed only from the ‘female’ parent (Anderson and Kohn 2007). More on these interesting points is discussed in the context of the genetic individual later in this chapter and in ►Chap. 5). For now, the important concluding point is that we see in heterokaryosis the ability of an organism to adjust the proportion of different sets of genes in response to environmental variation (e.g., available substrates). This is distinct from the formal mitotic-meiotic system of macroorganisms where the genotype (apart from somatic mutation) is continuous throughout the soma. The heterokaryotic fungus adapts genetically and physiologically literally as it grows. Successful heterokaryons, manifested by vegetative fusion and regulated nuclear exchange, as opposed to growth inhibition and cell lysis, are also evidence of nonself recognition systems in fungi, discussed later (Worrall 1997; Saupe 2000; Glass and Kaneko 2003). Finally, there are close parallels between this fungal system and cell/individual compatibility in colonial benthic invertebrates (e.g., Rosengarten and Nicotra 2011), a theme developed in ►Chap. 5.

Parasexuality Another distinctive attribute of the sexual process in fungi involves genetic recombination outside the usual sexual mechanisms. Some fungi, such as the opportunistically pathogenic yeast *Candida albicans* that were once thought to be asexual, have subsequently been shown to have a nonmeiotic parasexual cycle (Heitman 2006; Forche et al. 2008; Hickman et al. 2013). In the classic parasexual cycle there are four unrelated phases, each of which occurs relatively rarely (Pontecorvo 1946, 1956): (i) a heterokaryotic condition is established, as described above; (ii) diploidization (nuclear fusion) occurs giving a somatic, heterozygous, diploid nucleus; (iii) as growth ensues, the numbers of all nuclei increase by mitosis; in the diploid nuclei, mitotic crossing-over occurs between homologous chromosomes and occasional mitotic error can produce aneuploids; (iv) haploidization follows eventually as chromosomes are lost randomly in successive mitoses. Recombination results both from mitotic crossing-over as well as from the haploidization process. Parasexuality, in being a consequence of these uncoordinated, fortuitous events, is a process distinct from standardized sexual recombination. It cannot replace conventional meiotic sex as a means for recombining genes, and in any *one* generation contributes insignificantly to variation (Caten 1987). The irregular karyotic variation, however, may be advantageous in regulating physiologically important genes in *C. albicans*; and the parasexual cycle, in bypassing the conventional sporulation cycle of this pathogen, may contribute to its ability to live in prolonged association with its hosts as a commensal (Forche et al. 2008). Considerably more information is needed on the extent and significance of parasexuality in nature. Despite its apparent rarity, the process provides yet one more means for genetic recombination, especially in certain supposedly asexual organisms, and it illustrates how mitosis can play a role in genetic variability (Schoustra et al. 2007).

In overview, it is evident that the fungi are a genetically versatile transitional group that spans the gamut in means of transmitting genetic variability. They exhibit some of the orderliness (meiotic mechanisms) of the macroorganisms, together with haphazard variation mechanisms akin to those of the bacteria. Their idiosyncratic life cycles and strange nuclear processes and arrangements may reflect mechanisms to control access to the germline comparable to the evolutionary forces that led to historecognition systems in animals (Buss 1987) and are discussed in detail in ►Chap. 6.

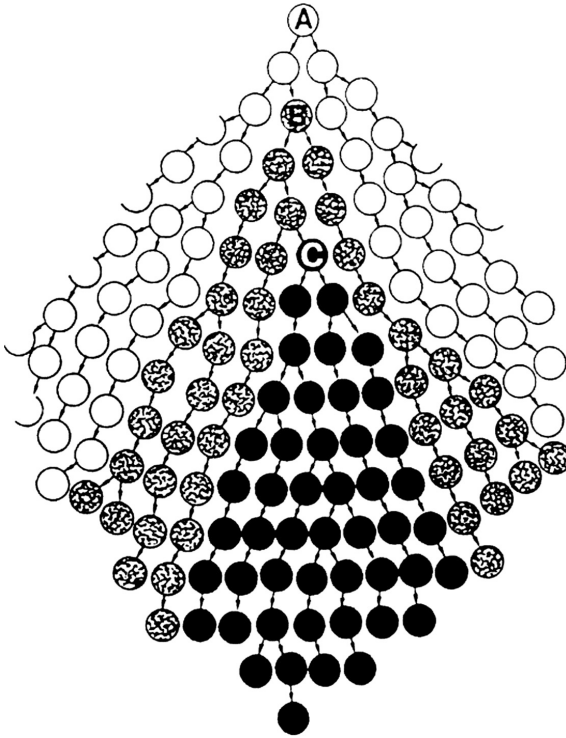
2.4 The Asexual Lifestyle

While most if not all extant organisms reproduce sexually, some are facultatively and others apparently obligately asexual. Asexuality is discussed here at some length because of its significant evolutionary implications and to set the stage for our later discussion of what constitutes a genetic individual. There are numerous variations on the theme of asexuality, each with its own terminology that is frequently inconsistent across disciplines (Normark et al. 2003; Jackson et al. 1985; Schon et al. 2009; Tibayrenc and Ayala 2012). The prevalent modes are: (i) **apomixis**, which strictly means that female progeny arise mitotically from unfertilized eggs. Its usage, however, is varied and is often equated with agamospermy or parthenogenesis, or alternatively equated with all forms of asexual reproduction; (ii) **vegetative growth**, wherein new individuals arise by fission or budding—as is exemplified by bacteria and yeasts, respectively; or fragmentation as in some plants and animals; and (iii) **automixis**, where eggs are produced meiotically but the meiotic products fuse. Clearly, the various modes can have somewhat different genetic consequences.

The entire set of individuals that descends exclusively (i.e., asexually) from a common ancestor is called a clone. As Milkman points out (1996) ‘exclusive’ means that *all* the genetic material in *all* the descendants originates with the common ancestor. The exact meaning of ‘clone’ varies by discipline and microbiologists usually have a more specific and practical context in mind than do botanists or zoologists; also, usage in microbiology is complicated by inconsistent semantics of multiple terms such as serotype, ecotype, sequence type, lineage, strain, and clonal cluster (for microbial examples, see Ørskov and Ørskov 1983; Anderson and Kohn 1995; Spratt and Maiden 1999; Henriques-Normark 2008; Tibayrenc and Ayala 2012, 2015; for macroorganisms, see Jackson et al. 1985; Hughes 1989). As one operational example from medical microbiology pertaining to *Staphylococcus aureus* genotyping, isolates that have identical nucleotide sequences at all of seven housekeeping genes are considered to belong to the same ‘clone’ and receive a unique ‘sequence type’. Those that are identical at five or more of the loci are known as a ‘clonal complex’ (Chambers and DeLeo 2009).

The word ‘clone’, used as a noun or more commonly today as a verb, has undergone considerable variation in meaning since its first application apparently in plant breeding in the early 1900s.³ Regardless of its current application in many subdivisions of biology, each with its own semantics, **one should not infer that all members of a clone are necessarily genetically identical**, despite that implication being drawn by some authors. While in the early stages of growth and depending on the mode of asexual reproduction (see above), all clonal members may be essentially identical, they diverge over time due to somatic mutation. The nested relationship of progressively diverging clones, most easily visualized for bacteria, is nicely illustrated by Milkman (1996; see also Spratt and Maiden 1999) (■ Fig. 2.7). However, some authors have defined clones more broadly. Writing mainly with respect to microorganisms, Tibayrenc and Ayala (2012, p. E3305; see also Spratt et al. 2001) argue that ... “*clonality does not mean the total absence of recombination, but that it is too rare to break the prevalent pattern of clonal population structure.*” Their key defining criteria for clonality are: (i) strong

3 Ostensibly, the first use of the word clone was in a horticultural context by H.J. Webber (1903, p. 502) where he defined clones (in that era spelled ‘clons’) as one subdivision of ‘variety’ specifically to mean “... groups of plants that are propagated by the use of any form of vegetative parts such as bulbs, tubers, cuttings, grafts, buds, etc., and which are simply parts of the same individual seedling”.



■ **Fig. 2.7** Clonality as a hierarchical or nested relationship with clones originating exclusively from a single common ancestor. This is most easily visualized as a progressively diverging series of bacterial cells dividing by fission, but is in principle applicable to all clonal organisms. Clone A is the oldest and progenitor of sub-clone B and eventually and indirectly of sub-clone C; B arises from A by (somatic) mutation and C diverges from B by further mutation. All three clones are closely related and ultimately traceable to cell A, but each has its own age and genetic structure. The process continues indefinitely. See text for complications and caveats. From Milkman (1996); reproduced by permission of ASM Press, Washington, DC ©1996

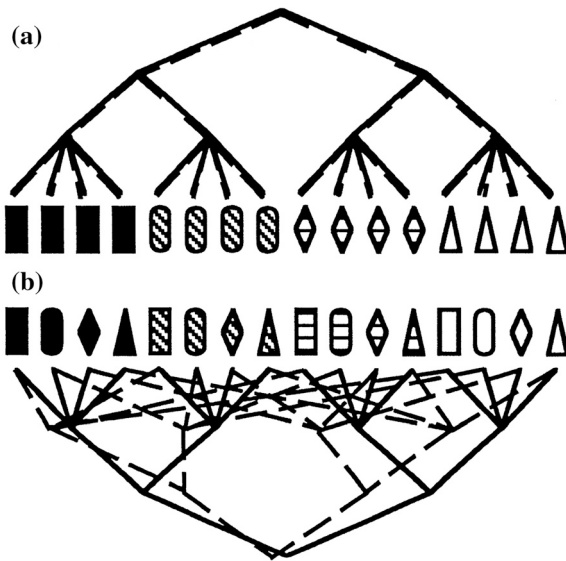
linkage disequilibrium together with (ii) clear phylogenetic signal. This would constitute “predominant clonal evolution” (Tibayrenc and Ayala 2015). Unfortunately, it is often not a simple matter to differentiate between episodes of mutation and recombination, either in microorganisms (Bobay et al. 2015) or macroorganisms (Ally et al. 2008). In prokaryotes, genotypic and phenotypic clonal divergence can occur rapidly (within hours to days) in culture, whether in heterogeneous (Rainey and Travisano 1998) or constant (chemostat; Maharjan et al. 2006) conditions (for general remarks, see Bobay et al. 2015). Thus it is the case both *in vitro* and *in vivo* that multiple clonal sub-lineages occur, each carrying at least one and likely many mutations. These subpopulations or ‘mutational cohorts’ compete in a phenomenon commonly referred to as ‘clonal interference’ (e.g., Williams 1975), and the population is not necessarily purged of variation by selective sweeps of a clearly superior line as once thought (Lang et al. 2013). Because of their shorter generation times and relatively much larger population sizes, microbial clones diversify faster in absolute time than clones of macroorganisms such as aphids or aspen trees.

In phylogenetic terms, lineages of asexual eukaryotes tend to be scattered among branches of their sexual relatives near the tips of phylogenetic trees. This implies that, in

general, asexuals have arisen relatively recently and are short-lived in evolutionary time, without having had the opportunity to diversify to a high taxonomic rank (Butlin 2002). Phylogenetic evidence, along with molecular genetics data attesting to the ancient and complex nature of the sexual process, suggest that asexuals have arisen sporadically from sexuals, rather than the other way round (Otto and Lenormand 2002; Rice 2002). Why asexuals generally do not persist is hotly debated. Stanley (1975) argued that most evolutionary change arises from speciation events and that asexual macroorganisms generally could not speciate rapidly enough over evolutionary and geological time to avoid extinction, thus accounting for the paucity of asexual clades. Another reason may be the relentless accumulation of deleterious mutations in a finite asexual population ('Muller's ratchet' noted earlier; Bell 1988; Barton et al. 2007). The rate at which the ratchet turns and its impact are subject to several assumptions. Depending on which ones are invoked, the theory actually predicts that asexuals would be eliminated quickly, slowly, or not at all (Normark et al. 2003). The common condition of polyploidy in asexuals has been interpreted as possibly insulating the organism from the deleterious effect of accumulating mutations (Otto and Whitton 2000; Pawlowska and Taylor 2004).

There have been numerous claims for ancient asexuals, occasionally referred to as "ancient asexual scandals" because, if true, the examples contradict conventional wisdom that asexual lineages cannot persist long (Muller 1964; Maynard Smith 1986; Bell 1988; Normark et al. 2003). As emphasized by Judson and Normark (1996), such records must meet all three components inherent in the term 'ancient asexual group,' namely that the lineage is: (i) descended from a common ancestral group (i.e., is monophyletic); (ii) 'ancient,' defined subjectively but commonly taken to be on a scale of geological time, an order of magnitude of millions or tens of millions of years; (iii) primitively asexual, i.e., that the group has remained asexual from its inception without interludes of sex. This latter attribute is the most difficult to establish and several taxa once assumed to be asexual have been shown to engage in cryptic sex. This includes fungi classified as arbuscular mycorrhizae (Glomeromycota), originally believed to be the eldest asexuals at ca. 400 million years (Kuhn et al. 2001; Croll and Sanders 2009; however, see Taylor et al. 2015). Males, hermaphrodites, and meiosis are unknown in a large metazoan taxon (Class Bdelloidea of the Phylum Rotifera), believed to be at least 35 million years old (Welch and Meselson 2000; Flot et al. 2013). Examples of other kinds of evidence used to infer not only asexuality but in some instances ancient asexuality are several, including: (i) the independent evolution of two alleles at any given locus. This increasing allelic divergence due to the accumulation of neutral or possibly adaptive (Pouchkina-Stantcheva et al. 2007) mutations is called the 'Meselson effect' (Welch and Meselson 2000; Butlin 2002); (ii) high taxonomic rank with abundance of species; (iii) phylogenetic congruence of gene genealogies; (iv) strong correlation among alleles at multiple, polymorphic loci (linkage disequilibrium) leading to the recovery of the same multilocus genotype through time and often over great distances; and (v) decay of sex- and recombination-specific genes (Tibayrenc et al. 1991; Taylor et al. 1999a,b, 2015; Normark et al. 2003). These and other tests vary in rigor, are subject to caveats, and the outcome may be subject to interpretations other than a conclusion of asexuality; for insightful discussion, see Taylor et al. (1999b) (■ Fig. 2.8).

At least in contemporary time and quite likely also in geological time, many lineages have effectively integrated alternating rounds of sexual and asexual reproduction into their life cycles. At the level of the individual or species, whether sexual or asexual reproduction dominates the cycle, or indeed occurs exclusively, depends on factors such as the local envi-



■ **Fig. 2.8** A simplified example of strictly clonal versus recombination reproductive modes and resulting phylogenies based on two hypothetical characters, shape and pigmentation (*shape* = rectangles, triangles, etc. symbols representing the four structures shown; pigmentation = degree of blackness or whiteness within the structures). In the clonal organism (a), recombination is absent; the entire genome remains intact through generations and occurs in few combinations as represented by the sub-clones showing constant association between degree of pigmentation and specific shape. In the recombining organism (b), pigmentation and shape are found in all possible combinations. Note, however, that genetic regions may be constantly associated for reasons other than clonality; likewise, lack of association of loci may occur for reasons other than recombination. From Taylor et al. (1999b); reproduced from *Clinical Microbiology Reviews* by permission of the American Society of Microbiology, ©1999

ronment and whether a compatible mating type also occurs. In an evolutionary context, this is perhaps particularly true of pathogens or parasites (Price 1980) and fungal pathogens especially (Andrews 1984; Heitman 2006). Sexual reproduction is often associated with adverse environments and its occurrence in some facultatively sexual fungi is probably a specific instance of the broader phenomenon known as ‘condition-dependent’ or ‘fitness-associated’ sex reported in various taxa (see Hadany and Otto 2009). Tsai et al. (2008) quantified the occurrence of sexual and asexual rounds in populations of the wild yeast *Saccharomyces paradoxus* and found that a sexual cycle occurs about once in every 1,000 asexual generations.

Generally speaking, trade-offs are evident in a sexual/asexual cycle, where production of the sexual spore form typically takes significantly longer (weeks vs. days) but may be more resistant to desiccation, whereas the asexual form is produced in much greater abundance. In the case of plant pathogens, timing of the life cycle phases is typically exquisitely linked to susceptible phenological stages of the host. Repeated rounds of asexual reproduction allow the pathogen to ‘track’ the host in time and space; regular episodes of sex (for pathogens of plants, these typically occur during the over-wintering or dormant phase) allow for generation of novel genotypes to respond to selection pressure of the evolving host. Taylor et al. (1999a) suggest that fungi also may be thought of as mosaics of recombining and clonal populations: the sexual populations to be found on wild, heterogeneous hosts where the recombined fungal genotypes are generated and then move to genetically uniform

agricultural hosts, where asexual populations cycle. Heitman (2006, 2010) and Taylor et al. (2015) review several interesting cases, including that of the opportunistic human pathogen *Cryptococcus neoformans*. Originally believed to be exclusively asexual, this yeast is now commonly referred to by its sexual state (teleomorph), *Filobasidiella neoformans* (Webster and Weber 2007, pp. 660–665). It inhabits trees, soil, and pigeon guano worldwide. However, opposite-sex mating between mating types *a* and α has been found recently in restricted habitats in India and sub-Saharan Africa, but likely occurs much more widely. Moreover, same-sex mating (self-fertility) also occurs and generates genetic recombinants that pose a hazard for the immuno-compromised AIDS population in this area of the world.

2.5 Somatic Variation, Heritable Variation, and the Concept of the Genet

The idea that the genetic individual or genet is the central entity in which mutational and recombinational events are expressed was introduced in ►Chap. 1. To recap very briefly, genets are classically viewed as the developing products of zygotes; they arise from a sexual process and therefore represent new genetic entities; in a sense they are independent genetic colonizations of a landscape (Harper 1977). Following our review in this chapter of sexuality/asexuality and the mechanisms of genetic variation, let us now return to the genet concept in a more detailed fashion. This is important, not only because ‘the genetic individual’ is referred to repeatedly in this book, but also because of its major influence in evolutionary biology.

The utility of the genet concept hinges on the premise that although genetic variation can occur in somatic cells, such variation cannot be transmitted to progeny. This view, propounded most forcefully by August Weismann in the late 1800s, can be stated more formally as: (i) the zygote produces somatic cells mitotically and germ cells meiotically; (ii) genetic variation developing during ontogeny cannot be inherited; and hence (iii) heritable variation is expressed only in the zygote or during meiosis in the formation of gametes (Weismann 1892; summarized by Buss 1987, p. 13). **If true, this so-called Weismannian doctrine has enormous implications: it restricts evolutionary change to a matter of selection among individuals**, as Buss (1987) insightfully develops in his book. So then, to what extent is the genet a valid and useful common denominator in phylogenetic comparisons?

First of all, it should be reemphasized that somatic variation occurs in all organisms and rates are at least as high and typically substantially higher than germline rates (earlier discussion and Lynch 2010). They are presumed to be a significant source of phenotypic and genotypic variation in plant populations (Silander 1985; Klekowski 1988) and in clonal, modular animals (Hughes 1989; more on this in ►Chap. 5).

Secondly, there is no doubt that somatic variation can affect the life of the organism. Probably the most dramatic evidence of this is that the main changes that lead to various cancers involve somatic mutations (Griffiths et al. 2015). The cells in most forms of cancer have aberrant chromosomes (e.g., deletions, inversions, translocations, aneuploidy) as well as numerous point mutations (ranging from a few to more than 1,000; Vogelstein et al. 2013). Accumulating somatic mutations also form the basis of one of the theories of aging (see ►Chap. 6). Other sorts of somatic change may be neutral or beneficial, either in allowing the organism to adapt to biotic or abiotic challenges (e.g., generation of antibody diversity, acquisition of acquired immunity), or by concomitant adjustment to specific genotypic

alterations. Recently, the history of somatic mutation accumulation within individual neurons of the human brain has been traced by single-cell sequencing (Lodato et al. 2015). Remarkably, each neuron (which lives and remains transcriptionally active for decades) has its own unique genome as a result of as many as ~1,580 single nucleotide variants (SNVs), among other genetic changes. These mutations appear to arise during transcription, unlike the standard case of errors being introduced during DNA replication. Highly expressed genes were enriched for the SNVs.

The extent to which somatic variation can enter the germline, which is the real issue in evolutionary terms, depends on the ontogenetic program of the organism. It is ‘the real issue’ because, as seen above, while somatic genetic changes can be devastating to the individual, possibly even causing death, **they are limited to that individual, not the lineage.** For dipterans, as illustrated by *Drosophila*, it would be highly improbable for somatic variants to enter germ cells. The totipotent lineage in *Drosophila* is restricted to only the first 13 nuclear divisions per generation (Buss 1987, pp. 13–25)—a fleeting opportunity for the origin of a somatic variant. Similarly, in humans, germ cells established in the 56-day-old embryo remain sequestered for up to about three decades (Buss, op. cit. p. 100). Based on current evidence, both dipterans and humans come as close as any organism does to being a homogeneous genetic entity. That the period of accessibility to the germline is short for vertebrates has been confirmed elegantly in studies where foreign genes are introduced during early embryogenesis (Robertson et al. 1986; Jaenisch 1988). For instance, early embryonic cells can be infected with retroviruses in vitro and reintroduced to the embryo at the blastocyst stage of ontogeny. The infected cells contain integrated provirus that contributes to both the somatic and germ cell lineages, as confirmed biochemically and by the chimeric phenotype of the transgenic animal and its progeny. Infection of pre-implantation stage mouse embryos results in transmission to the germline, whereas infection at the post-implantation stage (between days 8 and 14 of gestation) results in transmission to the somatic but generally not to the germline (Soriano and Jaenisch 1986). Thus dipterans, humans, and mice are examples of a type of ontogeny where all cell lineages are determined early in ontogeny, known as **preformistic development** (Buss 1987). A correlate of this developmental mode is the absence of ramet production. (Ramets, also discussed in ►Sect. 1.3 of Chap. 1, are the asexual counterpart to genets and will be taken up further in ►Chap. 5.)

At the other extreme, an ontogenetic program known as **somatic embryogenesis** is characterized by absence of a distinct germline and the ability to regenerate a new individual from some tissues at any life stage (Buss 1983, 1987). Somatic variants can be transmitted to progeny either by the mutated cell lineage passing directly to the ‘new’ individual during the process of asexual fragmentation, fission, etc. or by the lineage entering the gametes (Otto and Orive 1995; Orive 2001). For instance, in the fungi, mutations arising in any tissue can be transmitted sexually or asexually. Because asexual reproductive rates are so high, favorable mutants, such as those containing virulence alleles (Clay and Kover 1996), can be rapidly increased through natural selection (Caten 1987). Thus, by clonal growth, fungi can evolve significantly by mutation in absence of recombination. Among some simple animals such as *Hydra*, the zygote divides to produce an interstitial and a somatic cell lineage. The former remains totipotent and mitotically active. By the time gametes are differentiated it is highly likely that somatic variation will have arisen in the forerunners of those cells. Likewise, corals are totipotent and in Buss’s words (1987, p. 107) ... “a 20,000-year-old reef coral had passed uncounted millions of fruit fly generations.”

Plants are a particularly interesting example of the somatic embryogenesis mode of development. As we discuss in ►Chap. 5, they grow by virtue of the activity of meristematic cells in their shoot and root apices, and additionally in some cases by lateral meristems encircling the axis. The meristems accumulate mutations over repeated cell divisions as the cell lineage increases. A practical consequence of such mutations is that horticulturists have exploited them for centuries to develop most varieties of fruit trees, potatoes, sugar cane, and bananas, not to mention countless vegetatively propagated ornamental and floricultural plants (Silander 1985). The spread and impact of a somatic mutation depends on many factors, including the strength of selection at the level of cell lineage; when the mutation occurs in the timing of the mitotic lineage; and the number of cell generations per individual generation (Otto and Orive 1995; Otto and Hastings 1998; Orive 2001). Long-lived and particularly large-statured plant species have higher mutation rates per individual generation than do short-lived species because of the greater number of cell divisions before gamete formation (Klekowski and Godfrey 1989; Schultz and Scofield 2009). A biological consequence of somatic mutation is that plants can develop as mosaics where one component, say a shoot, is genetically quite different from another. This implies that beneficial somatic mutations (e.g., resistance to parasites or insect grazers) could potentially spread easily, whereas at least some kinds of deleterious mutations would be inconsequential because the affected part could be shed (hence, it is argued, no increase in mutational load would occur) (Whitham and Slobodchikoff 1981; Gill et al. 1995). This variation has been hypothesized as being one way by which long-lived plants could contend with rapidly evolving pests and pathogens. The evidence is mixed (see ►Chap. 5 and Whitham and Schweitzer 2002; Folse and Roughgarden 2011).

Because of the totipotency of plant meristematic cells and the clonal aspect of development, somatic mutations in precursors of a floral lineage can be transmitted to gametes. As alluded to earlier in this chapter, these events happen occasionally and have been documented in the groundbreaking work with transposable elements of maize pioneered by Barbara McClintock (McClintock 1956; Fedoroff 1983, 1989). For example, if a genetic change occurs during the first embryotic cell division, a plant with genotypically and phenotypically distinct halves is created. Each half will go on to produce different gametes. If a similar change is delayed until ears form, two different sectors with correspondingly distinct kernels will develop. Indeed, the order of genetic events can be surmised from the timing in appearance of the sectors. **The important point here, however, is that somatic changes can be reflected in the gametes and ultimately zygotes.** Hence, somatic variation can not only alter the fitness of the carrier in which they arise, but they can, at least in some instances, most notably with modular organisms, be passed on to offspring produced sexually (Otto and Orive 1995; Pineda-Krch and Lehtila 2004; see ►Chap. 5). This mechanism extends the conventional forms of genetic variation discussed previously (►Sect. 2.3).

Microorganisms have always posed a challenge for the genet idea. As noted above (The Asexual Lifestyle), although members of a clone are essentially identical initially, they diverge genetically over time due to somatic mutation and other processes. For the genet idea to hold, it is not necessary for daughter cells in a mitotic lineage to be genetically identical. Rather, the complication for microbes is mainly that the nature, occurrence, and transmission of a genetic change is often haphazard and may occur outside the conventional sexual cycle. Formation of the bacterial recombinant is not tied to a particular divisional event, a morphological structure, or a characteristic life cycle stage such as reproduction, dormancy, or dispersal. The recombinant cells are frequently not even evident in a mixed population unless identifiable phenotypic traits such as auxotrophic markers are involved. Some eukaryotic microbes

(e.g., the ascomycete and zygomycete fungi) typically are diploid only very briefly, so the nature of the event triggering a new genet is not clear, unlike the usual case among plants and animals (for elaboration of this point see Anderson and Kohn 1995). Fungi, perhaps uniquely among organisms, tend to fuse upon contact. While such comingling tends to be restricted by vegetative incompatibility systems to close relatives, a single fungal thallus may exist as a genetic mosaic, with genetically different nuclei operating within a common hyphal cytoplasm (Peabody et al. 2000; James et al. 2008; Roper et al. 2013) or spore (Kuhn et al. 2001). As we have seen, by way of heterokaryosis and parasexuality, mutational and recombinational events can be expressed, transferred clonally, and exposed to natural selection independently of fertilization. The evolutionary implications of migration of new genes through an existing genet, followed by change in phenotype and outgrowth of a new genet, present complications to conventional modular theory, a topic taken up in detail in ►Chap. 5. This fungal situation does not arise with unitary organisms because the germ cells are segregated from the soma, and within other modular life forms this kind of gene migration would be rare, if not unique. Thus, **while genets can be visualized clearly for most macroorganisms, the concept must be applied somewhat abstractly for some and perhaps most microorganisms.**

The preceding foray into potentially heritable somatic variation is necessary because it documents that genetic variation occurs at many levels, including the cellular, as well as those of the so-called ‘physiological’ and ‘genetic’ individual. This challenges the dogma that the developing product of the zygote (by which is implied a single entity arising from gametic fusion) is **the unit** of variation. It means, on balance, that the concept of a genet is an ideal that is more or less approximated in various phyla. The unitary organisms, most clearly illustrated by the vertebrates, come closer than do modular life forms (many invertebrates, plants, fungi) in behaving as genetic individuals. As reviewed earlier in this chapter, molecular biology is showing that mobile genetic elements can move among chromosomes of a cell, among cells, and between the somatic and germ lines. One consequence of this fluidity is increased somatic variation and potentially a direct route from soma to gametes. Even in the case of unitary macroorganisms, the concept of the genetic individual must now be revised to reflect more flexibility and fluidity.

2.6 Summary

The principal general categories or agents of genetic variation are mutation, recombination, and regulatory (expression) controls. Mutations are the ultimate source of most variation in all organisms. Broadly speaking, the sources include errors in replication of the genome; errors in segregation of the replicated genome to the daughter cells; and modification of the genome by events such as transposition or errors in recombination. Mutation rates per base pair per replication event vary over orders of magnitude from viruses to eukaryotes, but mutations are roughly comparable across taxa when expressed as per genome per replication event. The inherent rate of sequence divergence over time for a given gene or protein among taxa tends to be approximately constant (the so-called ‘molecular clock’). Nevertheless, rates of phylogenetic change are far from constant, apparently governed largely by the ecological opportunity afforded the genetic variants.

Recombination, the mixis or rearrangement of nucleotides, is accomplished by general (meiotic assortment; crossing-over; segmental interchanges) and site-specific (e.g., retroviruses; plasmids; transposons; promiscuous DNA) mechanisms. The potential of site-specific

mechanisms to dynamically restructure the genome and to affect genetic expression is only beginning to be fully appreciated. Sexual recombination, the bringing together in a cell of genes from two genetically different sources, is one of the means by which recombination can occur. Sex is essentially ubiquitous among organisms and is usually but not always associated with reproduction, that is, the generation of offspring. For example, sex in bacteria is never an obligatory aspect of reproduction. Most if not virtually all extant preponderantly asexual creatures can also reproduce at least sporadically sexually, although the sexual stage may occur infrequently and in some locations may be absent. Apparent absence of sex in certain living things may simply be a consequence of insufficient observation or an overly restrictive concept of sex, e.g., where it is construed to exclude events (e.g., transduction, transformation, plasmid-mediated conjugation, anastomosis and resulting heterokaryosis, parasexuality) that are in effect sexual but do not involve the fusion of gametes. Retroviral gene transport in mammals is analogous to transduction in bacteria. Meiotic recombination in eukaryotes has an obvious parallel in conjugal transfer in prokaryotes.

Sexual reproduction arguably predated the asexual process. It may have arisen in a process akin to bacterial transformation or as a mechanism to repair DNA from which the recombination function then evolved. In conventional diploid organisms, sexual reproduction entails a dilution of 50% of the genome per generation. Given this, as well as other disadvantages relative to an asexual alternative, why sex has been consistently maintained is unclear. In evolutionary terms, it has probably been retained for several reasons (e.g., resetting genetic variation; avoidance of Muller's ratchet; host-parasite coevolution), which may well be different or of differing importance in the various phyla. If this is the case, efforts to find a singular role for sex are unlikely to succeed.

The most obvious evidence for gene regulation at multiple levels from transcription to protein activity is that cells of the same genetic constitution differentiate into various tissue types. Transcription of bacterial genes is controlled by various mechanisms, among them repressor or activator proteins. In eukaryotes, controls include the sequestering of portions of the genome into transcriptionally inactive regions (heterochromatin); existence of cooperative groups of regulatory proteins; methylation of DNA; RNA processing, transport, and degradation; and many other factors. Because of numerous controls on gene expression, it is not necessary to disrupt the coding region of a gene in order to render a change in phenotype. The importance of changes in regulatory as opposed to coding DNA is increasingly being recognized for its seminal role in plant and animal developmental biology, as well as in the phylogenetic diversification of eukaryotes.

Overall, microorganisms appear to have essentially all of the capability of macroorganisms in generating genetic variability, though adaptive evolution proceeds rather differently in the two groups. The major distinction seems to be that variation specifically in the prokaryotes is transmitted in relatively dynamic, unordered fashion, as opposed to the orchestrated manner, characterized by meiosis and gametogenesis typical of the eukaryotic microorganisms and of higher organisms in particular. Much of the genetic machinery and reservoir of variation for bacteria is nonchromosomal, residing instead in numerous kinds of accessory elements, classically the plasmids. Perhaps most significant is that the gene pool of distant relatives is tapped by horizontal transmission that introduces fundamentally new traits in a manner that apparently occurs rarely in eukaryotes. The fungi, as eukaryotic microorganisms, have a fairly fluid genome as exhibited by such processes or conditions as parasexuality, dikaryosis, and heterokaryosis. Thus, they can be viewed to be an intermediate group, spanning the gamut in variability-generating mechanisms, dexterity, and orderliness of transmission.

The concept of a genetic individual or genet originated in Weismann's doctrine, which viewed the zygote as the seat of all heritable variation and the germline as being insulated from the soma. It has long been recognized that somatic variation occurs and that this can markedly influence the carrier and potentially its clonal descendants. Whether somatic variation can be transmitted to offspring produced sexually depends on the ontogenetic program of the organism. In taxa where the totipotent lineage is strictly limited, as in higher animals, the chance that somatic variation can occur and be passed on to the developing germ cells is remote. Where cells remain totipotent and mitotically active, as in plants, fungi, and the less complex metazoans, transmissible somatic variation is probable (see modular organisms in ►Chap. 5). This has been demonstrated strikingly, for example, in the case of mobile genetic elements of maize. Thus, the genet is a much less discrete unit in some taxa than in others. Nevertheless, the evolutionary significance of somatic variation remains sketchily documented and controversial. Even more contentious is the extent to which there may be direct genetic feedback from the soma to the germline by a mobile gene (e.g., retrovirus) mechanism. What is clear and perhaps most important in evolution is that the *ability* to transmit somatic variability *is* to varying degrees heritable. Obviously, the genet must be a more fluid entity than was conceived originally, in part because of increasing awareness of the role of mobile elements in genetic rearrangement and expression.

The main conclusions from this chapter, which sets the stage for the rest of the book, are that: (i) All organisms possess several and in principle analogous means of generating and transmitting genetic variation on which evolutionary processes, in particular natural selection, can act. The principal differences are the relatively unordered (microorganisms) versus ordered (macroorganisms) manner in which the variation is transmitted. (ii) Because of their short generation times and large population sizes, microorganisms have higher evolutionary rates as species than do macroorganisms. (iii) With respect to broad comparisons of taxa, the occurrence of different ontogenetic programs means that in some cases somatic variation can be transmitted to the germline; different and in some cases multiple nuclear conditions occur during the life cycle; and the ubiquity of mobile DNA, all mean that the concept of the genetic individual (genet) must be more fluid than as originally conceived. As such it should be used guardedly in some circumstances (such as for the bacteria, fungi, and simple metazoans).

Suggested Additional Reading

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- Buss, L.W. 1987. *The Evolution of Individuality*. Princeton Univ. Press, Princeton, N.J. *The history of life as a transition between different units of selection.*
- Dawkins, R. 1982. *The Extended Phenotype: The Gene as the Unit of Selection*. Oxford Univ. Press, Oxford, U.K. *A continuation of Dawkins' stimulating examination, begun in "The Selfish Gene", of the levels in the hierarchy of life at which natural selection acts. The focus is on "selfish genes" and not "selfish organisms".*
- Grodzicker, T., D. Stewart, and B. Stillman (Eds.). 2016. *21st Century Genetics: Genes at Work*. 80th Cold Spring Harbor Symposium on Quantitative Biology. *Collected papers on genetic mechanisms and molecular biology in the areas of gene control, chromatin, genome stability, developmental biology, and evolution.*
- Otto, S.P. 2009. The evolution of sex: recent resolutions and remaining riddles. *A symposium organized by Sarah P. Otto. American Naturalist*, vol. 174 pp. 51–594. July 2009.
- Worrall, J.J. (Ed.) 1999. *Structure and Dynamics of Fungal Populations*. Kluwer Academic Publishers, Boston, MA. *An excellent synthesis of fungal biology by multiple authors.*

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