# CHAPTER 6

DNA Supercoiling

In Chapter 4 we explained how a DNA molecule must twist – or untwist – and curve, in order to carry out its various functions in biology. For example, DNA has to untwist near the start-sites of all genes, often at or near TATA sequences, so that RNA polymerase can unwind or separate the strands and construct new RNA strands according to the rules of Watson–Crick base-pairing. Similarly, DNA must untwist at all origins of replication, so that DNA polymerase can construct a new DNA strand in readiness for cell division. Lastly, DNA must curve around a variety of proteins in the cell. Some of these proteins help to package the DNA into a compact form, while others help to control the activity of some particular gene.

In Chapter 5 we went on to explain how DNA can twist and curve at the same time, so as to form long, regular supercoils or spirals. Some DNA molecules are intrinsically twisted and curved, on account of their base sequences; but others become twisted and curved only when they bind to certain proteins.

In the present chapter we shall take the subject of supercoiling one step further, by describing DNA molecules which are not curved either on account of their base sequences, or as a consequence of binding to proteins, but which coil through space nevertheless on account of *torsional stress.* And in order to provide a simple introduction to what is admittedly a rather difficult subject, let us first see how torsional stress can affect the shapes of some familiar household objects.

Figure 6.1(a) shows a piece of telephone cord. This sort of cord has a regular coiled shape because it has been manufactured with inbuilt curvature and twist. It coils through space naturally, like the intrinsically curved DNA molecules described in Chapter 5. Figure 6.1(b) and (c), on the other hand, show a piece of ordinary electric power
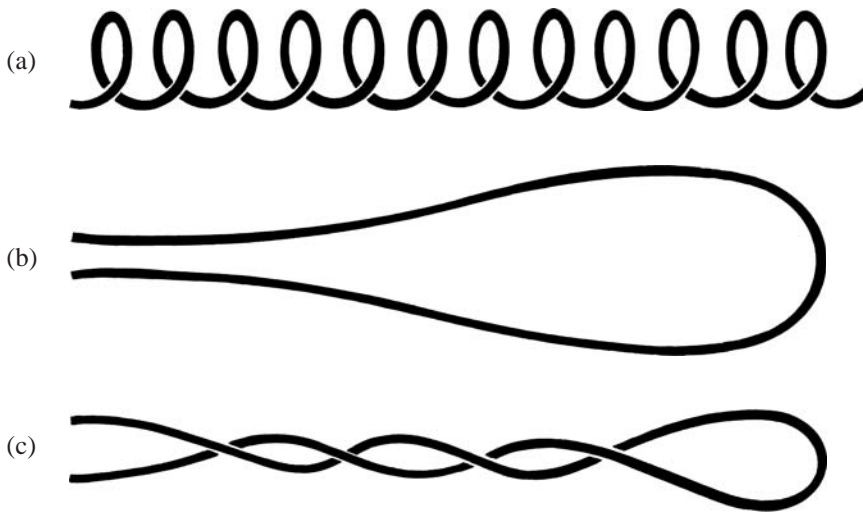
Figure 6.1 Everyday models for the supercoiling of DNA. In (a), a telephone cord coils through space naturally because it has inbuilt curvature and twist. In (b), an electric power cord has no inbuilt curvature or twist; but if you wind a few turns of twist into it, as in (c), it will cross over itself approximately once for every 360° turn of twist that you introduce.

cord. This kind of cord is manufactured without any inbuilt curvature or twist, and so it generally prefers to lie straight across a floor or table, or else in the kind of gently curving, broad loop sketched in Fig. 6.1(b). But if you take one end of this electric power cord in each hand, and wind a few turns of twist into it, then it will adopt the kind of shape shown in Fig. 6.1(c): the cord will cross over itself roughly once for every 360° turn of twist that you introduce. If you then release either end of the cord from the constraints of your hand, and shake it a bit, the free end will rotate in a reverse sense to eliminate the added twist; and the cord will return again to the uncrossed shape of Fig. 6.1(b).

Most DNA molecules, even though they are not intrinsically curved, can coil through space in the manner of the electric power cord shown in Fig. 6.1(b) and (c). It is very easy for a long DNA molecule to lose or gain a few turns of twist. This can happen, for example, by its binding to certain proteins, or by its unwinding locally during the synthesis of RNA; and if the two ends of the DNA are not free to rotate, then even a small change in twist can cause the path of the DNA to coil through space. Furthermore, there are several ways by which the two ends of a DNA molecule can be prevented from rotating. For example, the two ends can be joined with one another to form a closed circle of DNA, as shown in Fig. 6.2(a) and (b). Here, the ends of our telephone cord and electric cord have been joined into

(a)   (b)
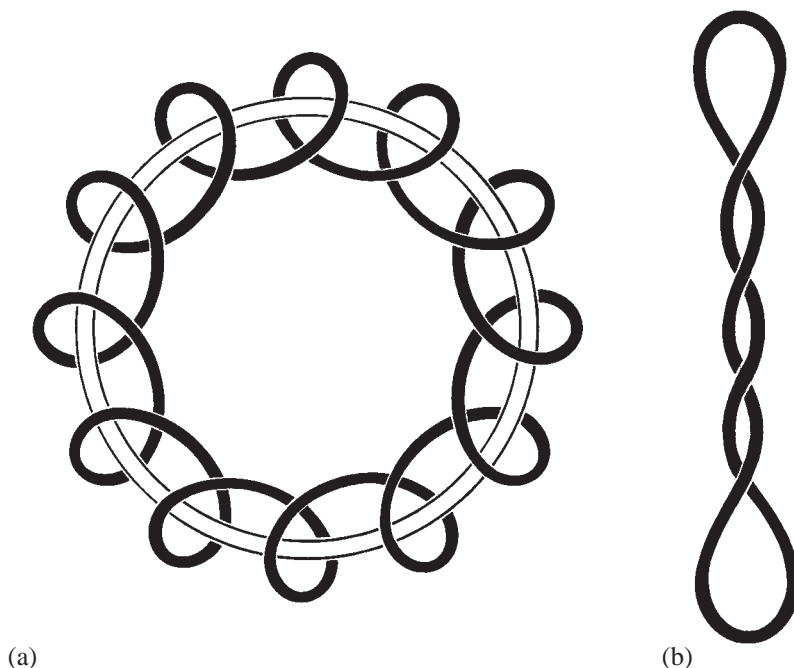
Figure 6.2 Two general varieties of DNA supercoil. In (a), the DNA coils into a series of spirals about an imaginary toroid or ring (shown here by open lines); and so this kind of wrapping is known as 'toroidal'. In (b), the DNA crosses over and under itself repeatedly; and so this kind of wrapping is known as 'interwound'.

shapes which are complex and yet 'circular', in the sense that they are endless. There are many examples of circular DNA in living cells.

Another example of end-restraint is shown in Fig. 6.3. Here, a long piece of linear DNA has been divided into a series of loops, and the two ends of each loop are constrained from rotation where they attach to some sort of supporting structure at the base of every loop. This kind of looped-linear arrangement is thought to be typical of the chromosomal DNA molecules which are found in higher organisms. We shall say more about this in Chapter 7. Both kinds of DNA, the circular and the looped-linear, will form supercoils upon any internal change of twist, because their ends are not free to rotate. In fact, there exists in our cells a variety of enzymes called 'topoisomerases', as mentioned in Chapter 4, that can cut the DNA temporarily so as to make a free end, and thereby relieve some of the effects of supercoiling. Without such enzymes, the DNA would get tangled about itself during normal cell function, and so it could hardly act as the genetic material. A full discussion of these highly complex enzymes is beyond the scope of our book, however; so the
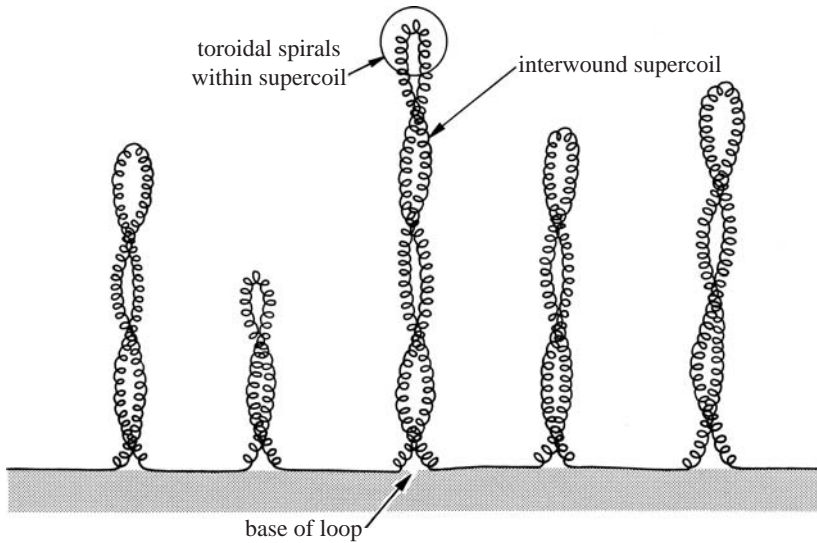
Figure 6.3 The division of a long, linear DNA molecule into loops generates end-restraint at the base of every loop, if the two ends are attached to some support or 'scaffold'. This kind of looped-linear arrangement is thought to be typical of the chromosomal DNA found in higher organisms.

reader should consult some of the references listed at the end of this chapter to learn more about them.

In any case, once we know that the ends of a DNA molecule are fixed, then we can identify two different kinds of supercoiling, which are epitomized by the two shapes shown in Fig 6.2(a) and (b). The circular DNA in (a) consists of a series of open spirals that wind around an imaginary ring, or toroid;[1] this kind of supercoiling is known as 'toroidal'. The circular DNA in (b), in contrast, winds above and below itself several times, and this kind of supercoiling is called 'interwound'. In practice, real DNA supercoils may contain portions of both the toroidal and interwound geometries. Thus, where certain parts of the DNA are highly curved, on account of either the base sequence or due to wrapping around a protein, one may find toroidal structures, since the DNA in a toroidal super-coil is highly curved throughout. Alternatively, if such curved portions of the DNA are not very long, they may locate themselves at the two strongly curved end-loops of an interwound supercoil, as shown at the top and bottom in Fig. 6.2(b). Sometimes the interwound and toroidal geometries may occur together, as in the looped-linear DNA which is shown schematically in Fig. 6.3. On a small scale, within any loop, the coiling is toroidal on account of the wrapping of DNA around protein spools; but on a large scale, over the full length of any loop, the structure is interwound. You

often see this kind of arrangement in telephone cords, if people habitually rotate the handset.

In general, supercoiled DNA has the shapes seen in Fig. 6.2 because it either has more turns of twist, or fewer turns of twist, than the underlying, relaxed, right-handed double helix from which it is made. DNA with more than the natural number of turns is known as *overwound*, while DNA with fewer than the natural number of turns is known as *underwound*.

Is there any way in which we can tell, by looking at pictures such as those shown in Fig. 6.2, whether the supercoiled DNA is overwound or underwound? And can we say by how many turns the DNA is overwound or underwound? Oddly enough, the second question is easier to answer than the first. Recall that we said above, in relation to the electric power cord of Fig. 6.1(b) and (c), that the cord crosses over itself approximately once for every 360° turn of twist which is wound into it. This idea is rather general, and it may be applied also to both of the supercoils of Fig. 6.2. Thus the supercoil (b), which contains four crossovers, is either underwound or overwound by about four turns, while supercoil (a) contains 12 crossovers, and is underwound or overwound by about 12 turns. Yet it is not so easy to decide whether these supercoils are overwound or underwound; before we can say anything on that point, we must learn more about the theory of supercoiling, as described below.

When circular DNA molecules are isolated from the cells of our bodies, or from bacteria, they are generally found to lack one turn of twist for every 17 turns of stable, right-handed double helix. Thus, they are said to be underwound, or negatively supercoiled, by about 6%. DNA molecules which contain *extra* turns are not found in Nature, except under special circumstances; and we shall explain below why that is the case.

It might seem unlikely that one could ever arrive at a single scheme of explanation that would account for the shapes of all possible varieties of DNA supercoil, whether toroidal or interwound. Nevertheless, such a scheme has been invented by the mathematicians James White and Brock Fuller. It is based on the branch of mathematics known as 'topology', which concerns itself with how things change shape when they go from one form to another. That is just what we want here, because DNA supercoils have lots of different shapes even though they are all made from the same kind of right-handed double helix.

We shall explain the application of topology to DNA supercoiling in two steps. First we shall deal with interwound structures, and then with toroidal ones. You will see when we have finished that we have actually used the same scheme in both cases.

Figure 6.4 shows a series of five, closely related, circular[1] DNA molecules. Two are in the form of open circles or simple rings, while three are in the form of interwound supercoils. In all cases, the DNA has been drawn as if its relaxed, stress-free form were a long rubber rod of square cross-section – recall Fig. 5.2(b) – with one face painted black. For example, the open circle in Fig. 6.4(a) is entirely black on one side. This means that it has exactly the same twist as relaxed, linear DNA of the same length; it is neither underwound nor overwound. Above each molecule in Fig. 6.4 we have added two symbols: Tw for 'twist' and Wr for 'writhe'. To a first approximation, Tw tells by how many turns the rubber rod twists as it goes once around the circle, and Wr tells how many times the rod crosses over itself within any molecule. Thus, Tw = 0 for the open circle in Fig. 6.4(a), because there we can see the black face of the rod everywhere; and Wr = 0 because the rod does not cross over itself.

Figure 6.4(b) shows an open circle with three extra turns of twist. This twisted configuration can be obtained from the open circle of Fig. 6.4(a) by cutting the rod in one place, inserting there three full turns of twist and then closing it up again. It is easy to confirm that the open circle (b) contains three extra turns of twist, because the black face of the rod now shows at three locations as you go around the circle. You can also see that this new twist is right-handed, because the rod rotates in a clockwise sense, like a corkscrew, as you go around the circle. Accordingly, open circle (b) has been labeled as Tw = +3: the positive sign indicates that the extra twist is right-handed, i.e. in the same sense as that of the underlying DNA double helix. Wr = 0 again here, of course, since the rod in Fig. 6.4(b) does not cross over itself.

Now, if you make a model of the twisted circle (b) from a rubber rod, or from a long leather belt, you will find right away that this shape is unstable. Thus, if you hold the twisted ring firmly down onto a table it will look like (b), but if you let go it will collapse quickly into one of the other forms, usually into (d) or (e).

In each of the forms (c), (d), and (e), the DNA crosses over itself at least once, and the value of Wr shown above each drawing records the number of crossovers: 1 for (c), 2 for (d), 3 for (e). Note that for each new crossover, the circle loses one turn of twist. Thus, Tw decreases from +3 in (b), to +2 in (c), to +1 in (d), and finally to 0 in (e). You can confirm the value of Tw recorded in each drawing by counting the number of times the black face appears and disappears as you go around the circle. It is this decrease in Tw which provides the driving force for the rod to collapse spontaneously from shape (b) to (e). For most rubber rods, or leather belts, or DNA molecules,

Tw = 0	Tw = +3	Tw = +2	Tw = +1	Tw = 0
Wr = 0	Wr = 0	Wr = +1	Wr = +2	Wr = +3

Lk = 0   Lk = +3

(a)   (b)   (c)   (d)   (e)
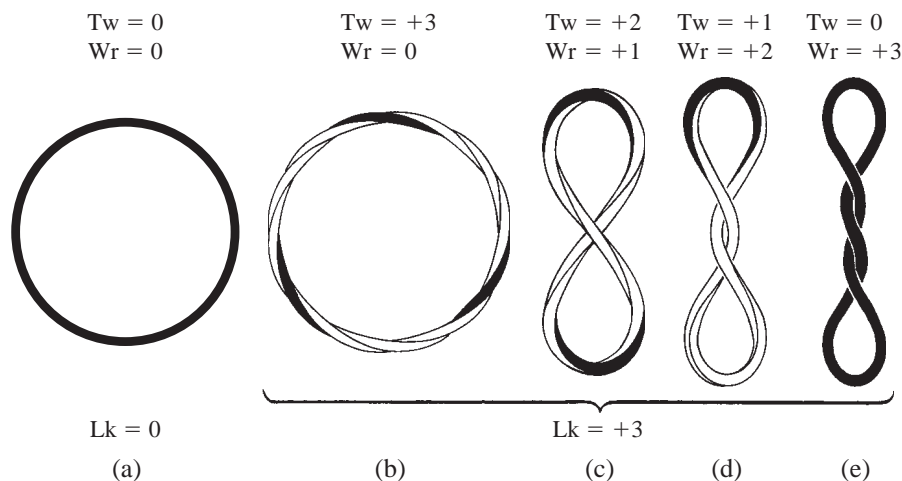
Figure 6.4 Five closely related circular DNA molecules: (a) and (b) show open circles, while (c), (d) and (e) show interwound supercoils. The DNA in its stress-free, relaxed form is drawn as a rubber rod of square cross-section, with one face painted black.

it is easier for the rod to cross over itself repeatedly than to alter its twist: the rod responds to the overwinding by *writhing* rather than by *twisting*. One could imagine, perhaps, that a very special rod could be constructed that would behave in the opposite way, preferring instead to remain in shape (b) rather than going to shape (e). Such a rod would be easy to twist, but hard to bend. However, most real rods and DNA molecules prefer configuration (e) to (b).

You may have noticed that the sum of Tw and Wr remains constant at +3 for all of shapes (b), (c), (d), and (e) in Fig. 6.4. This is no accident; and indeed it exemplifies a result of general significance. It can be shown to hold true for supercoiled DNA of any shape or size, provided that the meanings of Tw and Wr are defined rigorously (see below), and that the DNA double helix remains uncut and intact on both strands. For example, the sum (Tw + Wr) changed from 0 to +3 when we cut open the circle (a) and added three turns of twist; but then it did not change after that, in any of (b), (c), (d), or (e), because we did not cut the circle again.

Because the sum of Tw and Wr does not depend on the exact shape of the circle, but only on the intactness of its two DNA strands, mathematicians have given this quantity a special symbol, Lk, meaning 'linking number':

$$\text{Tw} + \text{Wr} = \text{Lk}.$$

Lk is known as a linking number because it is closely related to the number of times that the two sugar–phosphate chains of DNA wrap

around, or are 'linked with', one another. In this chapter, we have taken DNA in its relaxed state as the reference point for counting Lk. Thus Lk = +3 tells us that the DNA has three *more* double-helical turns than it would have in a relaxed, open-circular form. In general, Lk measures the total excess or deficit of double-helical turns in the molecule. So, when we say that a DNA molecule is 'overwound by three turns' we mean, precisely, 'Lk = +3'. Note, in particular, that Lk can only be an integer, because the DNA can only join to itself by some integral number of turns. (However, if a collection of DNA molecules in solution happens to adopt a series of integral values of Lk such as −19, −20, −21 and −22, which are closely related in energy to one another, then one could say also that the mean Lk = −20.5 for the group as a whole.)

Do Tw and Wr have to be integers also, or can they be real numbers such as +0.5 or +2.5? All of the pictures in Fig. 6.4 show values of Tw and Wr that are integral, but this is not necessarily so in all cases. For example, one could make an interwound supercoil with Tw = +0.5, Wr = +2.5 by rotating the lower lobe of supercoil (e) through an angle of 90° relative to the upper lobe. Then it would lie midway in shape between (d) and (e), which is satisfactory physically but hard to draw. A further untwisting of the lower lobe by an additional 90° would convert Fig. 6.4(e) all the way to (d), and yield Tw = +1.0, Wr = +2.0. So we see that neither Tw nor Wr needs to be an integer.

We have reached a point where we need more precise definitions of twist and writhe than those which we have been using so far. Let us consider twist first. In Chapter 5 we introduced a special twist t, measured in units of degrees per base-pair, to account for the long-range coiling of DNA through space. Thus $t = 0$ for a plane curve, but t is negative for a left-handed spiral, or positive for a right-handed spiral. The twist Tw as described here is very closely related to the twist t of Chapter 5. To find the value of Tw, we can simply take the sum of t values for all of the base-pair steps in the circular DNA, and then divide the total by 360°, in order to express the result in units of helical turns. Sometimes we do not have to use this complex definition if we wish to find the value of Tw. For example, we can evaluate Tw by inspection for each of the drawings in Fig. 6.4. But for more complicated shapes, in which the value of Tw is not an integer, it would be necessary to do the calculation in full.

Next let us consider *writhe*, which we have thought of in terms of the number of times the rod crosses over itself. The crucial point about Wr is that it is a measure of the shape of the DNA as a three-dimensional curve through space. Previously we counted the number of crossovers of the DNA in a single view in order to estimate Wr. All we need to do to get Wr accurately is to count the number of
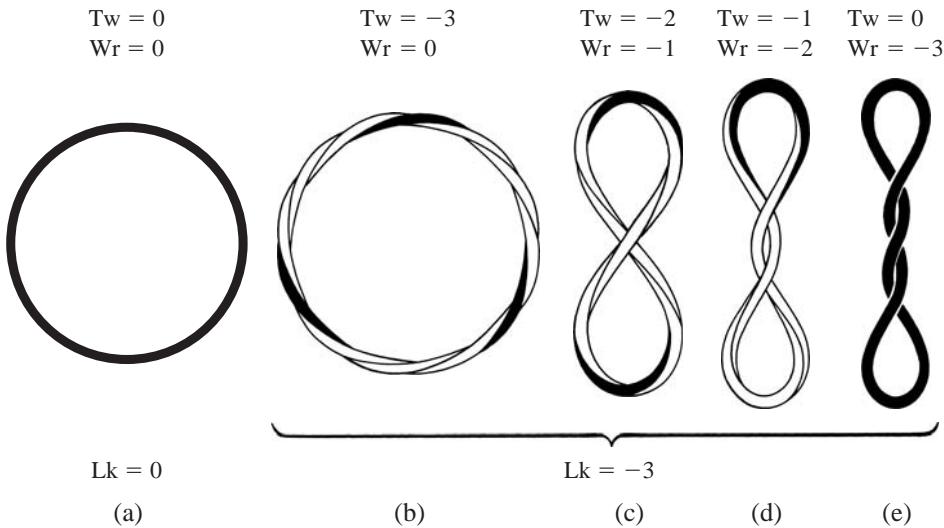
crossovers that can be seen in many different randomly chosen views of the structure, and then take the average of all of these to get the actual value of Wr. This is not a hard concept to grasp, if we think of taking a large number of snapshots of the DNA as it tumbles randomly through space, due to thermal motion. In practice, however, this may not be such a straightforward procedure, for in some views there can be many crossovers, some of which will cancel each other out: see Exercise 6.3. We shall avoid such difficult ideas in the main part of this chapter, so that only the specialist need worry about them.

Now the diagrams in Fig. 6.4 that we have been studying so far were drawn for positive Lk, i.e. for overwound DNA. You may recall that DNA in living cells is normally not overwound, but rather is underwound, and so its value of Lk is negative. Therefore, in Fig. 6.5, we have provided a corresponding set of pictures for negatively supercoiled, underwound DNA. You can see by comparing the two figures that the twist is now left-handed; for example, Tw $= -3$ and counterclockwise in Fig. 6.5(b). Also, the way in which the DNA crosses over itself (or Wr) is subtly different in Figs 6.4 and 6.5. Thus, the DNA crosses over itself in a left-handed fashion in Fig. 6.4(e) to give Wr $= +3$, but in a right-handed fashion in Fig. 6.5(e) to give Wr $= -3$. In fact, the handedness of the crossovers in any interwound supercoil enables you to say definitely whether the DNA is underwound or overwound, simply by looking at a picture. For open circular DNA, where there are no crossovers, you have to look at the twist of the rod to see whether the DNA is overwound (clockwise twist) or underwound (counterclockwise twist). The drawings in Figs 6.4 and 6.5 should serve as reliable references for further study.

At the beginning of this chapter we described two general classes of supercoil, known as interwound and toroidal. So far we have investigated only the interwound form. But now that we are familiar with the meanings of Lk, Tw, and Wr, it should be a relatively easy matter to analyze the shape of a toroidal supercoil, such as that shown in Fig. 6.2(a).

The best way of proceeding might be to examine in detail one small portion of a toroidal supercoil, say a segment of two superhelical turns. Figure 6.6(a) and (b) show how two such toroidal, superhelical turns can be generated by the collapse of a highly twisted piece of DNA. In diagram (a) we have drawn a twisted ribbon, with one of its sides shaded. It represents a piece of DNA which has been underwound by two turns. The ribbon is attached at either end to a block, so that its two ends cannot rotate: this device enables us to consider a small piece of circular DNA in isolation. For this piece of DNA you can see that Tw $= -2$ and Lk $= -2$, since Wr $= 0$: there are no crossovers in any perspective.

| Tw = 0 | Tw = −3 | Tw = −2 | Tw = −1 | Tw = 0 |
| Wr = 0 | Wr = 0 | Wr = −1 | Wr = −2 | Wr = −3 |

Lk = 0   Lk = −3

(a)   (b)   (c)   (d)   (e)

Figure 6.5 Five circular DNA molecules as in Fig. 6.4, but now with a linking number Lk of the opposite sense. These DNA molecules are underwound, with Lk negative, while the ones in Fig. 6.4 were overwound, with Lk positive.

In Fig. 6.6(b) we have moved the two blocks closer together, so that the twisted ribbon can collapse into part of a toroidal coil, like the coil made by a snake as it lies in the grass. The ribbon now makes two flat, left-handed turns: you can verify that they are left-handed by doing a simple experiment with a short piece of a leather belt, or a strip of paper. In this configuration both coils are almost planar, so Tw ≈ 0, and the linking number has not changed from its previous value of −2. Thus we find that Wr = Lk − Tw = −2. The main point which emerges from this exercise is that two flat, left-handed toroidal coils have a writhing number of −2. Thus, in going from Fig. 6.6(a) to (b), the twist of the DNA has been transformed into writhe.

Let us now return to the toroidal circle of Fig. 6.2(a). Its coils are certainly left-handed, but they are not so flat as those of Fig. 6.6(b). If we imagine a simple transformation by which the entire toroid is pushed inwards, so that its radius becomes smaller, then the coils will become flatter. In such a case the writhing number can be assigned as −2 for each pair of coils, just as in the example above; and so Wr = −12 for the entire molecule. On the other hand, if the supercoil were to be pulled out to a much larger radius, so that it became a simple, open circle, then it should have Wr = 0, and Tw = −12. In the configuration shown, Wr will lie somewhere between these two extremes, and closer to −12 than to 0; so perhaps Wr = −10.

You may have noticed in our pictures that a *left-handed* coil in the toroidal form of Fig. 6.2(a) gives negative writhe, while a *right-handed*